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An ecological and experimental study of
sediment-benthos interactions in a polluted estuary

Volume 1

By

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(B.Sc., M.Sc.)

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Samira
1985

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GENERAL SUMMARY

GENERAL SUMMARY

Section I

General description of the two major sampling sites

1. Animal abundance and animal biomass

Abundance and biomass were measured from three small and three large cores collected from Langbank and Ardmore sediments.

At Langbank, ^{Corophium}L. volutator was the most abundant species and made up 85% of the total number of animals. In terms of biomass, ^{Nereis}L. diversicolor was the ~~most~~ dominant species (~ 91%).

At Ardmore, ^{Pygospio}L. elegans was the most abundant species and made up 92% of the total number of animals. ^{Pygospio}L. elegans and ^{Arenicola}L. marina were the dominant species in terms of biomass forming 44% and 39% respectively of the total.

2. Bacterial counts

Viable counts of bacteria were determined using three types of nutrient media (nutrient agar (a freshwater medium), bacto-marine agar 2216, and teepol-lactose agar (a coliform medium)).

Samples from the top 1 cm surface sediment from Langbank and Ardmore were used. Bacteria colonies were counted 3, 5, 7, 10, 14 and 21 days after inoculation. Colony forming units (c.f.u.)/g of dry sediment were calculated from plates containing between 30 and 300 colonies.

The number of bacterial colonies recovered from Langbank sediment were higher than from Ardmore sediment on all three media. In the definitive experiment, the highest number of bacteria occurred on bacto-marine agar 2216, followed by the freshwater nutrient agar and then the teepol-lactose agar.

3. Chemical factors

(a) Salinity

Ardmore water samples had higher salinity values than Langbank ones. The salinity of overlying water was higher than the interstitial water at both sites.

(b) pH and Eh of water

pH was higher at Ardmore than at Langbank. Eh of overlying water was higher than interstitial water at both sites.

(c) pH and Eh of sediment

pH of Langbank and Ardmore sediments increased slightly with depth. Eh was high at the surface, decreased at 5 cm, increased again at 10 cm and then remained relatively constant to a depth of 40 cm.

(d) Organic carbon

Organic carbon content of the three sediments was determined using the wet oxidation method. The organic content of Langbank and Ardmore sediments was higher than that of Rockware sediment ^(commercially available). Ashing and acid-cleaning dramatically reduced organic carbon in all three sediments. Ashing was more effective in removing organic carbon than acid-cleaning. After ashing and acid-cleaning, both Langbank and Ardmore sediments contained significantly more organic carbon than Rockware sediment. Sediment particles were stained before and after cleaning to show the amounts of organic material present on particle surfaces.

4. Physical factors

(a) Shear strength

At Langbank, sediment shear strength increased progressively with depth. With Ardmore sediment, shear strength increased to a depth of 45 cm then decreased.

(b) Particle size analysis

Ordinary and cumulative plots were made of the particle sizes from all three sediments, and parametric analyses conducted using Inman's (1952) method. Parameters calculated were phi median diameter (Md_{ϕ}), phi mean diameter (M_{ϕ}), deviation measure (σ_{ϕ}), skewness (α_{ϕ}), second skewness ($\alpha_{2\phi}$) and kurtosis (β_{ϕ}).

The kurtosis, skewness, sorting (s.d.) and mean particle size of the three sediments showed that: (i) Langbank sediment was a leptokurtic, near symmetrical, moderately well sorted, medium sand; (ii) Ardmore sediment was a very platykurtic, fine skewed, well sorted, fine sand; and (iii) Rockware sediment was a mesokurtic, near symmetrical, very well sorted, fine sand.

Section II

Chemical and physical effects on sedimentation

1. Preliminary experiments

Preliminary sedimentation experiments were conducted to find the best sampling time and volume. The definitive method developed from the preliminary experiments was as follows: 42.61 ± 1.301 g of dried sediment ($\cong 55.36$ g wet sediment) and 500 ml of synthetic sea water were placed in a 500 ml stoppered glass measuring cylinder. The cylinder was inverted three times by hand before taking a sample. Two millilitre pipette samples were removed at 5, 10, 20, 40, 60 and 120 seconds from the centre of the water column at 10 cm depth.

2. Effects of organic material on sedimentation

Organic material was removed from Langbank, Ardmore and Rockware sediments by ashing and acid-cleaning (see Section I-3,d). This had a profound effect on sedimentation. Sedimentation of particles increased as their organic content decreased.

A linear relationship was found between \log_{10} (organic carbon) (x) and \log_{10} (suspended sediment) (y). These regression lines showed two major points. Firstly, there was a clear linear relationship between the amount of organic material on sediment particles and suspended weight. Secondly, the slopes of the regression lines increased with increasing time (except at 120 seconds) and was probably caused by the decreasing size of particles in suspension as time increased.

3. Effects of dryness, temperature and salinity on sedimentation

Wet sediments settled much more slowly than previously dried sediments. Sedimentation occurred most rapidly at 20°C. Sedimentation at 10°C was slightly slower than at 5°C. The most rapid sedimentation occurred at 0% salinity and the slowest sedimentation at 100% salinity.

4. Stokes' Law

Effects of organic material, salinity and temperature are interpreted in terms of Stokes' Law.

5. Field implications

Field implications of the effects of organic material on sedimentation are also discussed.

Section III

Effect of animal secretions on sedimentation

1. Effect of different types of secretions on sedimentation

Effects of benthic species secretions from Langbank (C. volutator and N. diversicolor) and Ardmore (P. elegans, S. armiger and A. marina) were tested on sedimentation of their natural and of Rockware sediments.

In general, secretions decreased sedimentation compared with control sediment with no secretion, except for the secretions of S. armiger which had no effect. These effects differed between the species. Secretions of A. marina followed by those of N. diversicolor had the greatest effect at 5 and 10 seconds, and secretions of N. diversicolor followed by those of C. volutator had the greatest effect at 40, 60 and 120 seconds. This means that the secretions of A. marina mainly reduce the sedimentation of coarse particles, the secretions of C. volutator mainly reduce the sedimentation of fine particles, but the secretions of N. diversicolor reduce the sedimentation of both fine and coarse particles. It follows that under field conditions resuspended particles bound together by secretions of the different species will be carried different distances depending on their particle size.

Scanning electron microscope photomicrographs showed that the secretions of each species were recognisably different.

2. Effect of enzymes on animal secretions

The digestive effect of six enzymes (α -amylase, hyaluronidase, lipase, lysozyme, pepsin and trypsin) were tested on secretions of C. volutator, N. diversicolor, P. elegans and A. marina. The enzymes had different effects. Comparisons between the enzymes showed that the most effective enzyme was α -amylase followed by pepsin, lipase, lysozyme, hyaluronidase and trypsin. This means that the secretions are mainly polysaccharides. Comparisons of the effects of the enzymes showed that the secretions of A. marina were the most resistant, followed by those of P. elegans and those of N. diversicolor and C. volutator, which were the least resistant. This may be important ecologically; it may mean that secretions of A. marina are the most resistant to bacterial enzymic degradation and will therefore last longer under field conditions, while

the converse will be true of the secretions of N. diversicolor and C. volutator.

3. How enzymic digestion of secretions alters the effect that the secretions have on sedimentation

Particles bound together by secretions and then digested with enzymes were tested in sedimentation experiments. Two controls were used - a top control (sediment with secretions but no enzymic digestion) and a bottom control (sediment with no secretions). The top control had the slowest sedimentation and the bottom control the fastest. Sediments containing enzymically digested secretions were intermediate - the more effective the digestion the closer they were to the bottom control.

4. Refinement of the sedimentation procedure

The five-second sedimentation readings in the secretion experiments (mg dry sediment/ml) were significantly higher than the theoretical values expected from an even distribution of the sediment at the beginning of the sedimentation experiment. Experiments were therefore conducted with secretions of C. volutator and N. diversicolor to find out why this was so.

The most important point demonstrated was that secretions caused greater weights and larger particles to be found towards the middle of the column. In the control sediment (no secretions), there was a gradual increase in particle sizes and weights towards the bottom of the water column. The field implications of these results are important. Larger sediment particles will settle more slowly when bound together by secretions of C. volutator and N. diversicolor and therefore will be transported further than sediment particles containing no animal secretions.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

I. Estuaries and their sediments

Estuaries are semi-enclosed bodies of water connected freely with the open sea and within which a measurable dilution of sea water by fresh water occurs (Pritchard, 1960). Estuaries represent a meeting place between fresh water - as run-off from the land - and the sea. Consequently, the estuarine environment is more extreme, and undergoes more violent fluctuations than the open sea or fresh water (Green, 1968; McLusky, 1981; Meadows and Campbell, 1978; Perkins, 1974).

Estuaries are often thought of as sediment sinks where sediment entering is trapped and transported by water currents (Davis, 1983). Sediment transported in estuarine and coastal waters is derived from three principle sources: (1) the bed of the continental shelf area; (2) that carried by inflowing rivers; and (3) dumping by man (Perkins, 1974).

Water circulation within estuaries depends on tidal range, vertical mixing between fresh and sea water, and the bottom topography (Meadows and Campbell, 1978). Tidal currents are powerful agents of sediment erosion and transport (Channon and Hamilton, 1976). On the boundary of the fresh water and sea water, mixing of the two water wedges takes place. The stronger the mixing of water, the stronger is the sea water current on the sediment bottom in the upstream direction of the river (Reineck and Singh, 1980). The height and energy of waves influences the depth to which sand is disturbed (Grant, 1981; Green, 1968; Perkins, 1974). The slope of the sea bed and the mouth of the estuary may affect the projection of the tide into the estuary and its travel up the river. The bed of the estuary which rises progressively causes an increase in the height of the tidal wave by retarding the front of the wave (Green, 1968).

In many estuaries, the inflowing tide moves to one side while the outflowing river moves on the other; at least during the early part of the tidal cycle (Green, 1968). Later the rising tide may push back the river water over the whole width of the estuary, or it may penetrate as a tongue of sea water while the fresh water continues to flow out over the top. There will be a certain amount of mixing during the tidal cycle and this varies from one estuary to another (Dyer, 1979; Green, 1968; Perkins, 1974; Reineck and Singh, 1980). Salinity is a good indicator of estuarine mixing and the pattern of water circulation (Dyer, 1979; Murray et al., 1975). Salinity or the amount of salt present in water influences its density (Green, 1968; Meadows and Campbell, 1978; Perkins, 1974) and the density of the water will affect the rate of sinking of particles which are present in it (Green, 1968).

The dominant factor in sediment transport is water currents (Briggs, 1977; Dyer, 1979; Green, 1968; McLusky, 1981; Perkins, 1974). The resistance of sediment particles to movement by water currents is determined by the size and weight of the particles (Briggs, 1977; Green, 1968; McLusky, 1981). In general, larger or heavier particles have a greater resistance to movement than smaller particles and settle faster than small particles (Gibbs et al., 1971; Green, 1968; Hulsey, 1961; Kajihara, 1971; Kuchem, 1968; Perkins, 1974). The velocity of the current required to move and transport sediment particles from the bed of the estuary is the entrainment or critical erosion velocity (Briggs, 1977; Perkins, 1974; Postma, 1967). The morphology of sand deposits in estuaries is determined by the interaction of a number of variables, including tidal range, tidal currents, wave conditions and storm action (Green, 1968; Hayes, 1975). Flood and ebb currents show distinctly different velocities at a given location. Slack water conditions permit fine suspended sediment to settle out (Perkins, 1974; Postma, 1967) whereas throughout much of the flood and ebb cycles erosion is dominant

over deposition (Davis, 1983). Despite this, estuarine mud flats are generally considered to be depositional environments (Anderson et al., 1981). The first section of my thesis describes some aspects of the physical, chemical, and biological environments of the two estuarine intertidal sites from which animals and sediment were collected for use in Sections II and III. In the second section of my thesis I describe some physical and chemical effects on sedimentation that are important in estuaries (temperature, salinity, wetness, dryness and organic content).

Living organisms can significantly influence the composition of sediments through their biological activities such as feeding, movement and the production of secretions and faecal pellets (Berner, 1980; Green, 1968; Gordon, 1966; Nichols, 1974; Perkins, 1974; Reineck and Singh, 1980; Rhoads, 1974; Rowe, 1974; Tevesz et al., 1980). The third section of my thesis is concerned with biological effects such as these, and I describe experiments on the effect of animal secretions on sedimentation.

II. Recent studies on estuaries

A large number of studies have been conducted on estuaries. They cover a wide range of subjects related to the estuarine ecosystem including microbiology, chemistry, morphology, hydrodynamics, biology and geoengineering. These references are listed at the end of this section (p. 11). In addition, several excellent multi-author books have appeared within the last twenty years (Cronin, 1971; Lauff, 1967; Stevenson and Colwell, 1973; Wiley, 1976, 1978), as well as two excellent elementary texts (Kennedy, 1984; McLusky, 1981).

The most recent book on estuaries is entitled "The Estuary as a Filter" (Kennedy, 1984). Estuarine processes are analysed, including the following subjects:

- (i) physical - turbulence, mixing and circulation;
- (ii) geological - sedimentation, flocculation and bioturbation;
- (iii) chemical/geochemical - nutrients, metals, and organic matter;
- (iv) biological/biochemical - surface foam, microbes, sea grasses, and wetlands. In addition, the problems and potential of estuary management are also considered providing new insight and direction for future research.

List of some representative literature references on estuaries

| Author | Year | Topic(s) |
|--|---------------------------------------|---|
| Colwell and Kaper Christain and Wetzel Sieburth Stevenson and Colwell | 1978 1978 1967 1973 | Microbiology in estuaries |
| Cronin Hunter and Liss Krank Sholkovitz | 1975 (Vol. I) 1979 1981 1976 | Chemistry |
| Anderson <u>et al.</u> Boersma and Terwindt Bridges and Leeder Lambiase Hayes | 1981 1981 1976 1980 1975 | Hydrodynamics and morphology |
| Cross and Sunda | 1978 | Geochemistry |
| Peirce and Williams Postma Wiley | 1966 1967 1976 (Vol. II) | Mechanisms of sediment transport and sedimentation |
| Cronin | 1973 (Vol. II) | Geoengineering |
| Frostick and McCave Grant Rhoads <u>et al.</u> Rhoads <u>et al.</u> Winston and Anderson | 1979 1981 1977 1978 1971 | Effect of fauna and flora on the movement and chemistry of sediment |

List of some representative literature references on estuaries (continued)

| Author | Year | Topic(s) |
|---------------------|---------------|--|
| Barnes | 1974 | General, including biology, type of estuary, geomorphology of particle sizes, erosion, transportation and deposition, environmental factors and their interactions |
| Cronin | 1975 (Vol. I) | |
| Green | 1968 | |
| Lauff | 1967 | |
| McLusky | 1981 | |
| Perkins | 1974 | |
| Swinbank and Murray | 1981 | |
| Wiley | 1978 | |
| Wiley | 1976 (Vol. I) | |

III. The Clyde Estuary

The hydrography, geology and biology of the Clyde Estuary have been described by Collar (1974), Deegan (1974), and Smyth (1974) respectively. The estuary of the River Clyde comprises two distinct parts, an upper, shallow, drowned estuary and the lower Firth of Clyde, making a total area of over 2,500 km² contained in a series of deep glaciated basins separated by shallow sills (Anon, 1974).

In general, it can be described as a partially or well mixed estuary in terms of water circulation. As well as the River Clyde, three major tributaries discharge fresh water to the inner estuary. The River Kelvin enters at 3.2 km, the two River Carts at 10.4 km and the Leven at 22.2 km below Glasgow Bridge (Collar, 1974).

In comparison with other estuaries in the United Kingdom, tidal ranges are relatively small. The Admiralty Tide Tables quote mean tidal ranges at the entrance to the dredged channel as 3.08 m springs and 1.89 m neaps, rising to 4.11 m springs and 2.40 m neaps at Glasgow Bridge (Collar, 1974).

Input of sediment to the estuary occurs from several sources. Collar (1974) states that 87.5% of the annual inflow of sediment is river borne, 6.25% comes from spillage within the dredged channel and 6.25% from solids discharged at sewage works, although these percentages may have changed since Collar's publication.

IV Pollution in the Clyde Estuary

The estuary receives discharges of pollutants at various points along its length. Pollution in the Clyde has three main origins. First, domestic sewage and industrial effluent from urban areas carried by the River Clyde and its tributaries; secondly, the dumping of sludge and sewage off Garroch Head; and thirdly, the enrichment of the

Irvine Bay area through the discharge of nitrogenous effluents from industrial plants (Heath, 1974). Hence the major pollutant is organic material with some heavy metals and other substances. There are also the special problems of thermal pollution from power stations and the release of radioactivity.

The upper estuary receives an average input of $0.41 \times 10^6 \text{ m}^3/\text{day}$ of treated sewage and $0.59 \times 10^6 \text{ m}^3/\text{day}$ of untreated and partially treated sewage (Anon., 1974). The latter is discharged to the tidal waters. These flows may be compared with a daily input of $22.3 \times 10^6 \text{ m}^3$ of fresh water, of which $9.2 \times 10^6 \text{ m}^3$ is discharged via the Clyde, Cart, Kelvin and Leven. The lower estuary receives untreated and partially treated sewage from a population of 150,000 people estimated at $0.045 \times 10^6 \text{ m}^3/\text{day}$. During periods of low flow in the summer the estuary can become totally deoxygenated for a distance of over 20 km downstream of the tidal weir in Glasgow.

V. The biology of the studied species

The biology of the dominant species at the two sampling sites are as follows:

1. Arenicola marina

Phylum Annelida

Class Polychaeta

Family Arenicolidae

Arenicola marina (Linnaeus) or the lug worm is very common around British coasts and is the best known sediment eater among the polychaete worms (Green, 1968; McLusky, 1981; Newell, 1970; Schäfer, 1972).

A. marina is often abundant in muddy sand. It is usually found burrowing in sand from the middle shore downwards. It also occurs in

the Mediterranean (rarely), Atlantic, English Channel, North Sea and West Baltic (Campbell and Nicholls, 1976; Longbottom, 1970).

The species reaches lengths of up to 20 cm. The head bears an eversible proboscis covered by papillae. The body is cylindrical, with six swollen anterior segments without gills, followed by thirteen segments with gills. The posterior part of the body is less swollen (Campbell and Nicholls, 1976).

Arenicola is absent from tidal flats with coarse clean sand and with very soft mud, but in tidal flats with some mud it is present in densities up to $100/m^2$ or locally even exceeding this number in the Dutch Wadden Sea (as is reported by Cadée, 1976, from Bellkema, 1976).

Arenicola marina feeds by ingesting ^{Sand} and digesting any organic matter that may be present. A. marina lives in 20 to 40 cm deep burrows in tidal flats (Cadée, 1976), may remain in the same burrow for many months (Green, 1968; Schäfer, 1972). There is some variation in the form of the burrow depending on the type of sediment, but, in general, it is L-shaped with a tail shaft which continues downwards into a gallery with walls that are consolidated by secretions from the surface of the worm. The lower end of the gallery turns more or less horizontally and ends against the head shaft which is not open but filled with sand. As the worm ingests sand from the lower end of the tube, the sand above slowly sinks downwards, leaving a depression at the surface. At intervals of about 45 minutes the worm moves backwards up to the tail shaft and defaecates on to the surface of the sand, depositing cylindrical worm casts that are common on sandy beaches with a slight admixture of silt (Green, 1968). A. marina irrigates its burrow by pushing water through the burrow and this may also serve to increase the supply of available food (Cadée, 1976; Green, 1968).

Most populations spawn in autumn, with maximum spawning on neap tides in October, November or December. Two years normally pass before A. marina spawns for the first time, and before spawning about 40% of the adults die (Newell, 1948).

A. marina can tolerate reduced salinities down to about 8‰.

2. Corophium volutator

Phylum Arthropoda

Class Crustacea

Order Amphipoda

Corophium volutator (Pallas) occurs along the European coasts from Norway to the Adriatic, the Baltic Sea, the Black Sea and the Azov Sea (Campbell and Nicholls, 1976; Crawford, 1937; Stock, 1952; Segerstråle, 1959). In North America it has been recorded in the Bay of Fundy, and in Nova Scotia (Segerstråle, 1959). It is the commonest member of the genus Corophium occurring in Great Britain. C. volutator frequently occurs in high numbers in muddy sediments where population densities of up to 11,000 animals/m² have been reported (Spooner and Moore, 1940).

C. volutator is essentially a selective deposit feeder although the animal sometimes filters fine particulate matter from the water column while in its burrow (Fenchel et al., 1975; Hart, 1930; Meadows and Reid, 1966; Nielson and Kofoed, 1982). When behaving as a selective deposit feeder Corophium scoops up and shifts small quantities of mud with the gnathopods. Larger particles are conveyed to the mandible where they are crushed by molar processes and swallowed (Nicol, 1960). Smaller particles are retained by a fringe of setae on the gnathopods, sifted, and then transferred to the mandibles to be swallowed.

The animal characteristically constructs U-shaped burrows in the sediment to a depth of about 4 to 6 cm (Schäfer, 1972; Foster-Smith, 1978) using the pediform second antennae and the gnathopods (Meadows and Reid, 1966). Hart (1930) noted that the animal lines its burrows using a mucous secretion released from glands at the base of the second pereopods. The formation of permanent burrows is dependent on the particle size of the sediment, the adhesive properties of detritus, and primary films on sediment particles (Meadows and Reid, 1966). Meadows (1964a,b and c) studied the substrate preferences of the species and found that animals prefer fine sand to coarse and anaerobic to aerobic substrates. Meadows (1964a) has shown that destruction of the surface film by boiling with acid removes the attraction of the specific substrate.

The reproduction of C. volutator has been studied by Hart (1930), Fish and Mills (1979) and Watkins (1941). Two generations are normally produced per year. The young develop to the first moult within the brood pouch of the female before being released. On release, they burrow beside the parent burrow. Sexual maturity is reached at a length of about 5.5 mm whereupon the animals disperse to form new colonies. Campbell and Meadows (1974) demonstrated that adult animals are highly gregarious. C. volutator can survive as long as 16 days in tap water (Green, 1968).

3. Nereis diversicolor

Phylum Annelida

Class Polychaeta

Family Nereidae

Nereis diversicolor (O.F. Müller) (the rag worm) reaches lengths of up to 12 cm and occurs in the Mediterranean, Atlantic, English Channel, North Sea and West Baltic (Campbell and Nicholls, 1976). It is found in the middle shore down to shallow water, burrowing in sand or mud,

often in brackish conditions, reaching its greatest abundance in estuarine muds. Densities of up to 4000 animals/m² have been reported (Schäfer, 1972). It is very tolerant of low salinities (Smith, 1955) and has been found at salinities as low as 1‰ (Green, 1968). Although N. diversicolor can tolerate very low salinities it cannot live and breed in fresh water. The larvae cannot tolerate as low salinities as the adult.

Reproduction of the species has been studied by Dales (1950, 1951), Chambers and Milne (1975), and Green (1968). In the United Kingdom, N. diversicolor spawns in February as the water temperature rises above 5°C. Females release their eggs by rupture of the body wall and die after spawning (Green, 1968). Males release spermatozoa through nephridia and are generally less abundant than females during spawning. The eggs take one week to hatch after fertilisation. Newly hatched larvae do not enter the plankton but remain within the surface mud or within parental burrows. The young do not feed for about seven weeks until the yolk sac is absorbed. After reaching 4 mm in length the young begin to construct U-shaped burrows.

Nereis constructs branched burrow systems of tubes to depths of 10-20 cm or 40 cm in winter with many connections to the surface (Schäfer, 1972). The animal burrows using a bolting action in which the pharynx is used to make an initial depression in the sediment (Trevor, 1977). The body is then forced into the depression by a combination of undulating and peristaltic movements. The burrows are lined by a mucous secretion released from glands covering the entire surface of the epidermis (Schäfer, 1972). Peristaltic movements of the body press the secretions against the burrow walls so consolidating them.

Nereis feeds selectively on a variety of materials including algae, detritus, other annelids and Crustacea, but can also filter feed (Harley, 1950; Macginitie and Maginitie, 1968; Nicol, 1960; Perkins, 1958). Mucous threads from glands on the parapodia are attached to the walls of the burrow and shaped into a funnel by the setae. Undulating movements of the body then draw water through the funnel and at intervals the animal swallows the funnel with its entrapped food particles.

4. Pygospio elegans

Phylum Annelida

Class Polychaeta

Pygospio elegans reaches lengths of between 10-15 mm (Fauvel, 1923) and lives in tubes that are consolidated with sand grains (Schäfer, 1972). This species is found in the North Sea, English Channel, Atlantic, Mediterranean and Baltic (Fauvel, 1923). It feeds on detritus lying on the sea floor (Green, 1968; Schäfer, 1972). It collects its food with two long grooved tentacles. These tentacles are ciliated and are waved about in the water or moved across the surface of the sand in which the worm burrows. Small particles, including diatoms are collected in the grooves and transported towards the head.

The species sometimes occurs in enormous numbers in estuarine sands. Thamdrup (1935) found densities up to 20,300 animals/m² on the Danish coast. Kaestner (1967) stated that Pygospio lives in U-shaped burrows, in areas well supplied with diatoms, at densities of up to 20,000 animals/m². P. elegans like many other species of polychaete, builds dwelling tubes from sediment particles. The building materials consist of fine sand grains and various shell fragments (Schäfer, 1972). The 1 mm thick tubes extend to 9 cm depth. Sand grains in the burrow

lining are cemented together with mucus. In the absence of sand, the tubes are constructed from lumps of mud, detritus, or plant remains.

P. elegans penetrates estuaries to a salinity of 8⁰/oo and from observations on the population in the Gwendraeth Estuary it appears to be able to tolerate salinities as low as 2⁰/oo for short periods (Remane, 1958).

Female Pygospio may produce up to sixteen egg capsules in the breeding season. Each capsule is anchored to a sand grain by a thin thread. Each capsule contains fifty eggs, but only two to nine of these develop. The others survive as nurse cells which disintegrate and act as nourishment for the embryo (Schäfer, 1972). The small number of embryos that hatch as bottom crawling larvae are about 1.5 mm long (Kaestner, 1967).

5. Scoloplos armiger

Phylum Annelida

Class Polychaeta

Family Orbiniidae

Scoloplos armiger (O.F. Müller) reach lengths of up to 15 cm. The head generally lacks appendages, but possesses two eyes which are sunk deeply in the head. The body is made up of numerous segments (up to 200) bearing chaetae, of which up to twenty may be in the flattened thoracic region. The body is divided into two regions. The thoracic half is flattened and enlarged, and the abdominal half is long and cylindrical. Parapodia bear simple gills on the dorsal surface. Body colour is generally pink or orange. The species generally inhabits sand or mud, and occurs in the Atlantic, English Channel, North Sea, West Baltic and Pacific (Campbell and Nicholls, 1976; Green, 1968; Kaestner, 1967; Schäfer, 1972).

S. armiger eats mud in much the same manner as Arenicola, by extrusion and retraction of the proboscis. The worm ingests small organisms and fine detritus together with sand. S. armiger burrows through the sand of tidal flats. It does not have a permanent dwelling tube and rarely rises to the surface (Schäfer, 1972). While burrowing the worm constantly secretes mucus, but this does not consolidate the burrow walls. It thus creates digging trails which cause only minor disturbances to the sediment (Schäfer, 1972). Its faeces are not cohesive and thus have no definite shape.

S. armiger produces baton-shaped egg capsules which are anchored in the sand by a stalk. Fertilisation takes place in a jelly capsule surrounding the male and female (Marshall and Williams, 1972). Each capsule contains about a thousand eggs (Green, 1968). Scoloplos larvae hatch out of the egg and remain on the bottom (Kaestner, 1967).

VI. Plan of thesis

The work reported in this thesis is divided into three sections as follows.

Section I

In the first section, the general biotic and abiotic features of the two major sampling sites were investigated. This included animal abundance and biomass, bacterial counts and chemical (salinity, pH, Eh, organic carbon) and physical measurements (shear strength and particle size analysis).

Section II

In this section, the effect of several chemical and physical factors on sedimentation was studied. This included the effects of organic material, different salinities, different temperatures, wetness and

dryness on sedimentation. Some aspects of sedimentation theory and sediment transport are also explained.

Section III

In my last section, the effects of some benthic invertebrate species' secretions on sedimentation are described. The second part of this section includes the effects of six types of enzymes on animal secretions.

VII. Note on statistical analyses

Two-way analyses of variance were applied to the results from various experiments. Where the interaction factor was not significant the result of the two-way analysis of variance is presented with the text. If the interaction was significant, then a series of breakdown one-way analyses of variance and students' t-tests were applied. In this case, the two-way analyses have been placed in the Appendix, and the final one-way analyses and/or t-tests are presented with the text. Throughout my thesis, I have often used the term one/two-way anovars as an abbreviation for one/two-way analyses of variance. I have also used the following coding throughout:

| <u>Probability</u> | <u>Rating</u> |
|---------------------|---------------|
| $0.05 > P > 0.025$ | * |
| $0.025 > P > 0.01$ | ** |
| $0.01 > P > 0.005$ | *** |
| $0.005 > P > 0.001$ | **** |
| $P < 0.001$ | ***** |

SECTION I

GENERAL DESCRIPTION OF THE TWO MAJOR SAMPLING SITES

SECTION I

Introduction

The main object of the work reported in this section was to determine the main biological, microbiological, chemical and physical properties of the sediments at Langbank and Ardmore sampling sites. The chemical and physical properties of a pure quartz sand (Rockware sand) were also determined, since it was to be used in later experiments.

The biological properties measured included animal abundance and animal biomass at mid-tide level on both shores. The general biology of the dominant species at Langbank and Ardmore, Corophium volutator, Nereis diversicolor, Pygospio elegans, Scoloplos armiger and Arenicola marina, are described in the general introduction.

The microbiological properties that were measured at both sampling sites consisted of bacterial counts of the surface sediment using a seawater nutrient agar, a freshwater agar and a teepol-lactose agar that was diagnostic of coliforms.

The chemical properties included measurements of salinity, pH, Eh, and organic carbon. The physical properties included shear strength and particle size analysis of the two sediments.

Many of these properties are important for determining the distribution of benthic invertebrates (Cummins and Lauff, 1969; Macginitie, 1978; Meadows, 1964a,b; Meadows and Anderson, 1968; Meadows and Campbell, 1972; Rhoads and Young, 1971; Rees, 1975; Wieser, 1956, 1959; ZoBell and Feltham, 1942).

(I) Animal abundance and animal biomass

There are many studies on the abundance, biomass, distribution and diversity of a wide range of animal species in sedimentary environments. These can broadly be divided into studies on subtidal

species (Biernbaum, 1979; Johnson, 1971; Nichols, 1970; Rhoads and Young, 1971; Sanders, 1958; Young and Rhoads, 1971) and studies on intertidal species (Bloom et al., 1972; Brown, 1982; Cadée, 1976; Croker, 1967; Gray and Rieger, 1971; Holme, 1949; Longbottom, 1970; Stephen, 1930; Whitlatch, 1981; Woodin, 1974).

The species I have studied are all found intertidally, and my survey work was conducted in the intertidal zone. The intertidal zone is a very complex habitat because of the wide fluctuations in environmental factors such as wave action, dehydration, temperature fluctuations, salinity fluctuations, substrate characteristic competition and so on (Barnes, 1974; Green, 1968; Newell, 1970; Perkins, 1974).

For example, Whitlatch (1981) determined the diversity of some deposit-feeding species in intertidal sand and mud-flats of Barnstable Harbor, Massachusetts. He found that both particulate and bulk sedimentary characteristics were related to the diversity of the deposit-feeding species. Species richness was correlated with the amount of surficial sedimentary organic carbon, and species diversity with total particulate and food particulate diversity.

Another example is Woodin's (1974) study on the abundance patterns of some marine soft-sediment environment polychaetes in Mitchell Bay, San Juan Island, Washington. She found no correlations between the abundance of four large and numerically important species (Lumbrineris inflata, Axiiothella rubrocincta, Platynereis bicanaliculata and Armandia brevis) and physical factors. Her experimental laboratory studies and abundance data demonstrate the presence of biological interactions, including interspecific and intraspecific competition for space. This data showed that such biological interactions are important determinants of polychaete infaunal species abundance patterns

in soft-sediment environments.

(II) Microorganisms

Microorganisms in soil and sediments belong to many different groups in the plant and animal kingdoms (Alexander, 1977; Burges, 1958; ZoBell, 1946; Wood, 1965). On the plant side fungi, algae, bacteria and actinomycetes are the most numerous. The bacteria form a very heterogenous group of organisms which are difficult to classify. Algae and diatoms are widespread in the surface layers of most soils and restricted to mud and water depths penetrated by sunlight. On the animal side, protozoa, flagellates and minute metazoan organisms are widely distributed in marine bottoms, for example ciliates and nematodes may occur in very large numbers.

The microbiology of intertidal sediments has received considerable attention over the past thirty to forty years. For example, the presence of microorganisms on the surface of sand grains has been studied in some detail (Anderson and Meadows, 1965, 1969, 1978; Anderson, Boonruang and Meadows, 1981; Dale, 1974; Deans, Meadows and Anderson, 1982; Deflaun and Mayer, 1983; Meadows, 1965; Meadows and Anderson, 1966, 1968; ZoBell and Feltham, 1942).

Bacteria play an important role in food cycling by synthesising cell substances and by converting waste or dissolved organic matter into a particulate form which can be utilised as food by animals (Wood, 1965; ZoBell, 1938, 1946, 1973). In a sedimentary environment, microorganisms clearly influence the invertebrate population in providing an important source of nutrition (Christian and Wetzel, 1978; Fenchel et al., 1975; Kostalos and Seymour, 1976; Macginitie, 1978; Newell, 1965; Wood, 1965). The importance of microorganisms in habitat selection by both larval and adult marine invertebrates has

also been extensively studied (Gray, 1966; Gray and Johnson, 1970; Meadows and Williams, 1963; Meadows, 1964; Meadows and Campbell, 1972a,b; Wilson, 1955). In addition, microorganisms have been shown to produce chemically different binding agents (Aspiras et al., 1971). These various binding agents produced in situ confer different physical properties on soil aggregates. For example they can affect stabilisation (Aspiras et al., 1971; Bathurst, 1967; Frankel and Mead, 1973; Geoghegan and Brian, 1946, 1947; Ginsburg and Lowenstam, 1958; Haworth et al., 1946; Holland et al., 1974; Neumann et al., 1970) and conserve the water content of soil and sediments (Waksman and Martin, 1939). Changes in the physical properties of soil also tend to decrease run-off and erosion (Browning and Milan, 1944; Bathurst, 1967; Neumann et al., 1970; Waksman and Martin, 1939).

(III) pH and Eh in the environment

In sedimentary environments, Eh and pH are commonly interdependent (Friedman and Sanders, 1978). Eh is a measure of the electron concentration in solutions and pH a measure of the hydrogen ions or protons. Because electrons neutralise protons, many reactions depend on both Eh and pH. High values of Eh, representing a low electron content, are generally accompanied by low values of pH (high proton content) (Bass Becking et al., 1960; Friedman and Sanders, 1978).

The Eh of sediments, like the pH, can be measured either colorimetrically or electrometrically (Langmuir, 1971; ZoBell, 1946). In my study I used the electrometrical method to measure the Eh values of the two sediments and the water samples at the two sites (see Materials and Methods, p. 47). Eh and pH values in near shore and estuarine sediments have been investigated in a number of studies (Aller and Yingst, 1980; Anderson and Meadows, 1978; Bålgander and Niemistö, 1978; Fenchel, 1969; Fenchel and Riedl, 1970;

Howes et al., 1981; Moshiri and Crumpton, 1978; Pearson and Stanley, 1979; Sorokin, 1975; ZoBell, 1946).

For example, Anderson and Meadows (1978) studied microenvironments in marine sediments. They discussed in detail three samples (i) sand grains and attached microbial colonies; (ii) banding in sediments; (iii) invertebrate burrow linings. In their study on the banding in sediments, they observed dramatic changes in Eh values and in the visual appearance of intertidal and shallow marine sediments over short distances of a few mm. This was associated with the rapid alteration of benthic microflora. However, they did not mention any pH measurement in their study.

Another example is ZoBell's (1946) study on the redox potential of marine sediments. He stated that the redox potential of sediments may be used advantageously in the study and interpretation of the layering and chemical processes in unconsolidated sediments. Data based on more than 1,000 samples of bottom deposits indicate that each type of sediment has its own characteristic Eh and pH. ZoBell (loc. cit.) recorded redox potentials ranging from Eh +0.350 to -0.500 volt. He also reported that, the pH of the bottom deposits ranged from 6.4 to 9.5, most of them falling in the range of pH 7.5 to 9.0. The pH of most sediments was found to increase with depth. The Eh generally decreases with depth, that is, conditions were found to be more reducing with depth.

(IV) Organic matter

The organic matter in sediments comes from a very wide range of sources. Organic material can enter the sediment from the land (wind-blown animal and plant material), and from the water column in the form of detritus (Anderson and Malahoff, 1977; Barnes, 1974;

Böhm et al., 1980; Campbell, 1977; Friedman and Sanders, 1978; Gross, 1971; Lynch and Poole, 1979; Nedwell and Brown, 1982; Reineck and Singh, 1980; Siebold and Berger, 1982; Tissot and Welte, 1978). In addition, animal faeces, secretions from algal mats, and algal sheaths containing mucilage (Barnes, 1974; Campbell, 1977; Friedman and Sanders, 1978; Tissot and Welte, 1978) can be an important source of organic matter.

Organic matter in sediments or sedimentary rocks can be considered to be derived from either soft tissues or hard tissues (skeletal remains). Tissues and their decomposition products are primarily carbonaceous compounds, whereas skeletal remains may be composed of carbonates, silica or phosphate compounds (Gross, 1971; Hobson and Menzel, 1969; Siebold and Berger, 1982; Tissot and Welte, 1978). The type and amount of organic matter in the sediment is considered to be one of the most important factors controlling the sedimentary environment (Nedwell and Brown, 1982; Tissot and Welte, 1978).

Marine organisms in sediments obtain organic matter from their environments in different ways: by scraping the film of organic matter from particles, by swallowing the sediment grains and digesting the organic materials from their surfaces, or by filter feeding (Barnes, 1974; Cadée, 1976; Gordon, 1966; Green, 1968; Levinton, 1982; Longbottom, 1970; Meadows and Campbell, 1978; Nichols, 1974; Ott et al., 1976; Rhoads and Young, 1970; Schäfer, 1972; Yingst, 1976).

The amount of organic matter available in sediments can affect sediment turnover rates by deposit-feeding invertebrates (Gordon, 1966; Nichols, 1974). This turnover is itself important in determining the structural properties at the sediment-water interface (Rhoads, 1970) and in the transfer of dissolved materials from

sediments to the water column (Nichols, 1974). Browning and Milam (1944) studied the effect of different types of organic materials and lime on soil aggregation. They found that, the addition of organic materials to these soils improved some of the physical characteristics and affected the susceptibility of the soil to erosion.

There are many reagents, general procedures, literature references and equipment for analyses and estimations of carbon content of sediments and sedimentary rocks (Byers et al., 1978; Dean, 1974; Gross, 1971; Holme and McIntyre, 1984; Walkley and Black, 1934). The various techniques differ substantially in their simplicity, precision and reproducibility, freedom from errors, and type and cost of the analytical equipment required.

Some of the above studies included comparisons between different methods, and the overall consensus is that the wet oxidation method is a good general purpose one. I, therefore, measured the amount of organic carbon in the three sediments using the wet oxidation technique.

(V) Shear strength

The shear strength of a soil or sediment is its maximum resistance to shearing stresses (Capper and Cassie, 1976; Jumikis, 1962; Lambe and Whitman, 1979). The shear strength of a soil or sediment depends on many factors including water content, particle size, inter-particle binding, gravity, and cohesion (adhesion) and friction between particles (Bowles, 1978; Capper and Cassie, 1976; Friedman and Sanders, 1978; Jumikis, 1962; Prakash, 1981; Smith, 1981; Yong and Warkentin, 1966).

There are a number of geotechnical studies of near-shore and estuarine sediment in which shear strength has been measured (see

Discussion, p. 137 for references).

For example, Deans, Meadows and Anderson (1982) studied the physical, chemical and microbiological properties of intertidal sediments. They studied the changes in sediment properties that occurred during sediment collection. Marked changes occurred in all the properties. One of these properties was shear strength. They noticed a marked decrease in the shear strength value by a factor of 8 following sediment collection and mixing which was needed to produce a uniform sample for analysis.

A second example is that of Moore (1964) who studied the shear strength and related properties of sediment from deep sea cores. He observed that shear strength varied from 0.07 bar in the upper meter to 2.66 bars at a sediment depth of 136 m. In his study, shear strength was positively correlated with calcium carbonate content and the relative concentration of the clay mineral montmorillonite, whereas it was negatively correlated with porosity, the plasticity index, and concentration of other clay minerals. In addition, Moore observed that, except at one site (EM8-15), there was a negative correlation between shear strength and content of clay sized particles.

Trask and Rolston (1950) studied the relation between shear strength of sediments and their water content and grain size. Factors affecting shear strength to some extent are mutually inter-related. They showed that the three principle variables affecting strength are water content, grain size and mineral composition, and that these three are not independent variables. For example, water content of sediments varies inversely with grain size.

(VI) Particle size

The particle size of sediments varies enormously depending whether one is considering a muddy, sandy or shingle beach (Collinson and Thompson, 1982; Leeder, 1982). The particle sizes found on these beaches have major effects on the type and abundance of animals that are found there (Gray, 1974; Jansson, 1967; Levinton, 1982; Meadows and Campbell, 1978; Newell, 1970), and many marine invertebrates living in mud or sand are often limited to their substrate by particle size (Croker, 1967; Meadows, 1964; Morgan, 1970; Phillips, 1971; Teal, 1958; Wieser, 1956, 1959).

For example, the niche diversity in five sympatric species of intertidal amphipods (Crustacea:Haustoridae) has been studied by Croker (1967). His substratum preference experiments indicated a choice of cleaner-over more silty substrata for two intertidal species, Neohaustorius schmitz and Haustorius sp., but no preference by the three other species.

Meadows (1964) studied the preference of Corophium volutator for different particle sizes of sediment. He found that C. volutator preferred fine to coarse particles over a range of particle sizes when offered a choice.

A third example on the importance of particle size is that of Teal (1958) on the distribution of fiddler crabs in Georgia salt marshes. In a substratum preference study on fiddler crabs, Teal showed that, Uca minax and Uca pugnax preferred mud to sand, while Uca pugilator preferred sand to mud.

(VII) The two study areas

Three types of sediments were used in the experiments in this section (Langbank, Ardmore and Rockware sediments).

Langbank sediment was collected at about mid-tide level from an intertidal sandy/muddy beach at Langbank in the Clyde Estuary, Scotland (National Grid Reference $55^{\circ} 56' N$, $04^{\circ} 34' W$). Ardmore sediment was collected from mid-tide level on an intertidal sandy/muddy beach at Ardmore Point in the Clyde Estuary ($55^{\circ} 58' 23'' N$, $04^{\circ} 41' 7'' W$).

A section of the Clyde Estuary which includes both sampling areas is represented in Figure 1. A narrow central channel is bounded by extensive intertidal mud-flats which show an overall tendency to increased width in a downstream direction. The two sampling areas are circled. The first (Plate 1) is situated near the village of Langbank, south bank of Clyde Estuary. The second (Plate 2) is situated approximately four miles east of Helensburgh on the north bank of Clyde Estuary.

The macrobenthos population at Langbank is not diverse and there are only two abundant species, Corophium volutator and Nereis diversicolor. Macoma baltica is also present, but it is less abundant.

The benthic fauna at Ardmore Point is dominated by Pygospio elegans, Scoloplos armiger, Arenicola marina, Macoma baltica and Fabricia sabella. The following species are also present: Nereis diversicolor, Mytilus edulis, Littorina littorea, Cardium edule and Phyllodoceids.

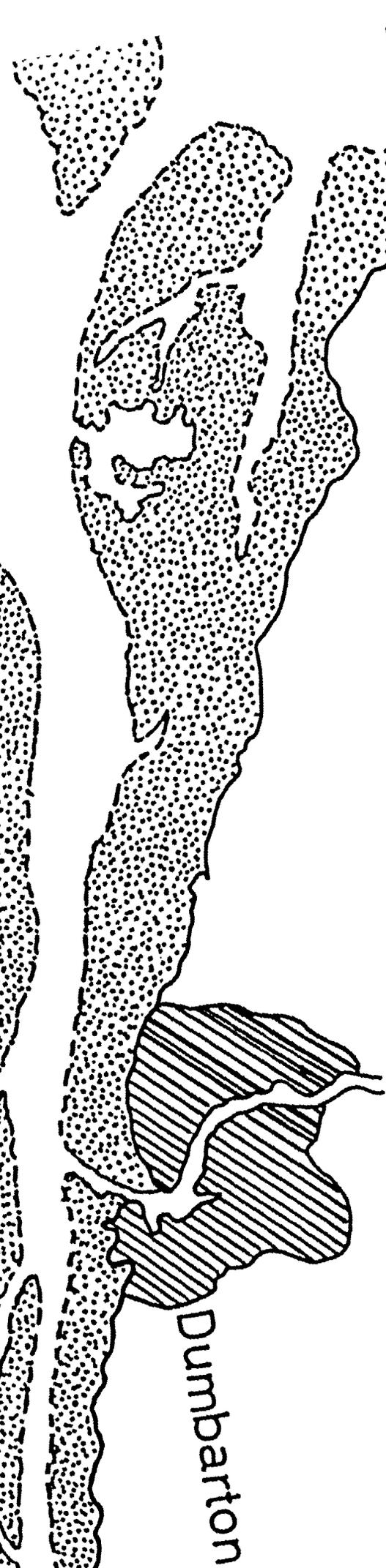
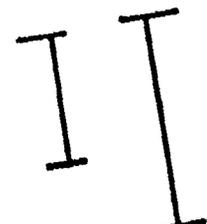
The third sediment used in this study was Rockware sediment. Rockware sediment was obtained from the Rockware Glass Co., Ltd, Irvine, Scotland. It is a naturally occurring pure quartz sand and was used in the sedimentation experiments.

Figure 1

A map of the Clyde Estuary, showing the two sampling areas.

Ardmore

Scale: 1 Inch = 1 Mile
1.6 cm = 1 Km



Port Glasgow

 : Sample site

Langbank
West ferry

Dumbarton

ERS
bridge

Plate 1

The mud-flat at Langbank. The pools^(a) and small rocks^(b) covered with sea weed are characteristic of the sampling area.

Plate 2

The sandy-mud-flat at Ardmore. Note the casts of Arenicola marina which were always abundant (arrowed)



1



2

SECTION I

Materials and Methods

The Materials and Methods in this section are divided into four parts. The first (I) gives the animal abundance and animal biomass (dry biomass); the second (II) gives the bacterial counts; the third (III) gives the chemical measurements (salinity, pH, Eh and organic carbon); the fourth (IV) gives the physical measurements (shear strength and particle size analysis).

The above measurements were carried out on the natural sediments (Langbank and Ardmore sediments). However, particle size analysis and organic carbon measurements were also conducted on Rockware sediment since this sediment was to be used in later sedimentation experiments.

Control, acid-cleaning and ashed Langbank, Ardmore and Rockware sediments were all used in the sedimentation experiments reported later in this thesis. Organic carbon measurements are therefore presented below on acid-cleaned and ashed as well as on the untreated controls sediments. Particle size analysis of all three sediments are presented for the same reason.

(I) Animal abundance and animal biomass

Animal abundance and biomass were measured ^(during may 1984) as follows.

1. Three small and three large core samples were collected on each shore. Large and small cores were used because rare species might not have been sampled by the small core and because very abundant species might have been too numerous to count in the large one. In practice neither of these two alternatives occurred (see Results). The diameter of the small and large cores were 8.13 cm

and 10.7 cm respectively. Core samples from each sediment were removed to a depth of 20 cm. Each core was divided into two equal depths (0-10 cm and 10-20 cm). Animals were removed from the sediments using a 500 μ m sieve. The animals were then transferred to a white enamel dish for counting. Animals were individually counted using a small paint brush to transfer them from one side of the dish to the other.

After counting, the animals were transferred to previously weighed metal foil boxes. Each species was placed in a separate box. The foil boxes containing the animals were placed in an oven at 60°C and weighed after 24 and 48 hours. The 24- and 48-hour dry weights were the same.

This procedure resulted in six abundance values and six biomass values for each species at each of the two sites and from each of the two depths - three from the big cores and three from the small cores (Appendix I - Tables 1-4, pp. 435 - 438).

The method of abundance and biomass determination was as outlined above for all species except Arenicola marina.

2. Abundance and biomass of Arenicola marina

Arenicola marina only occurred at Ardmore. Its abundance and biomass were measured as follows.

(a) Abundance

The number of casts in one square metre was counted at mid-tide level. Three different square metres were counted. The number of casts is taken to be the same as the number of animals (Cadée, 1976; Holme, 1949; Longbottom, 1970).

(b) Biomass

Twenty-five complete Arenicola marina were collected. Their wet weights were determined, and they were then transferred individually to previously weighed metal foil boxes. Boxes containing animals were placed in an oven at 60°C and weighed at 24 and 48 hours. The 24-hour weights were sometimes greater than the 48-hour weights. The 48-hour weights were therefore used in dry biomass calculations (Appendix I, Table 5, p. 439).

The wet biomass and dry biomass of A. marina were calculated by multiplying each of the three counts of number of casts/m² by the mean wet weight and the mean dry weight of the 25 animals (Appendix I, Table 6, p. 440).

(II) The bacterial counts

The materials and methods in this section are divided into five parts: the first (1) describes the media preparation; the second (2) gives the water content of both sediments; the third (3) describes sediment collection and treatments; the fourth (4) describes how plates were inoculated; the fifth (5) gives the viable counts method.

Two experiments on bacterial counts were conducted on each of the two sediments (Appendix I, Tables 10 and 11, pp. 444 - 445).

1. Media preparation

Three different media were used to culture bacterial colonies from both types of sediment.

(a) Nutrient agar

I decided to use this medium, because both of my sampling sites are situated in an estuarine area. Langbank lies close to the river discharge, whereas Ardmore Point is

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closer to the sea. Nutrient agar is a rich nutrient medium used principally to culture freshwater heterotrophic bacteria. It was prepared as follows.

Twenty-eight grammes of oxoid powder (CM3) was dissolved in one litre of distilled water. The mixture was thoroughly mixed and boiled to dissolve completely (Oxoid Manual, 1976).

(b) Bacto-marine agar 2216

This medium was used to culture heterotrophic marine bacteria, and was prepared as follows.

Fifty-five point one grammes of bacto-marine agar 2216 was placed in a conical flask and then one litre of cold distilled water was added. The mixture was heated to boiling point to dissolve the medium completely (DIFCO Manual, 1972).

(c) Teepol-lactose agar

The medium was prepared by adding the following ingredients to one litre of distilled water (Cruickshank et al., 1975, p. 125).

Twenty grammes of bacteriological peptone;
10 g of lactose; 5 g of sodium chloride (NaCl);
1 g of teepol; 25 ml of bromothymol blue solution
(1 in 500 solution); 9 g of agar. The mixture
was thoroughly mixed and heated to dissolve the
constituents. The pH of the medium was found to
be 7.5 as expected (Cruickshank et al., 1975).

(i) Sterilisation of media

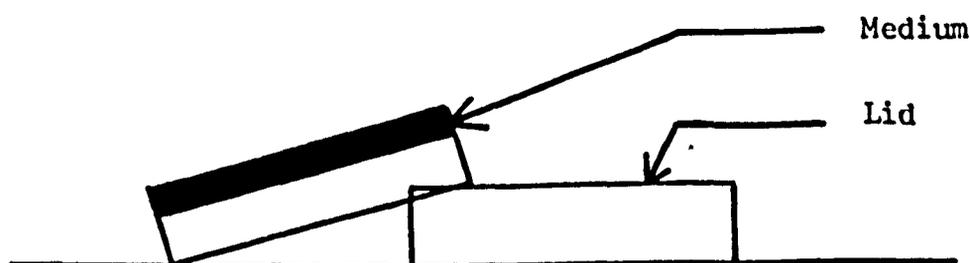
The nutrient and bacto-marine agar 2216 media were sterilised by autoclaving at 121°C for 15 minutes. The teepol-lactose agar medium was sterilised at 115°C for 15 minutes (Cruickshank et al., 1975).

(ii) Agar plates

Agar plates were prepared from all three media by pouring approximately 15 ml of the molten sterile medium into 9 cm petri dishes (Cruickshank et al., 1975; p. 99). Dishes were kept undisturbed until the medium had set.

(iii) Drying of plates

After plates have been poured, steam from the hot liquid medium condenses on the surface of the medium. This moisture is undesirable, and it is essential that the surface of the medium should be dry for spread plating. This surface moisture was removed by drying the plates on a previously sterilised flat surface at room temperature for 90 minutes. The surface was dry-wiped and then sterilised by wiping with an alcohol-dampened cotton wool pad three times before use. The way in which the plates were dried is shown in the following diagram (Cruickshank et al., 1975; p. 99).



Care was taken to avoid disturbing the air near the surface during plate drying. All the above precautions were found to be necessary to ensure that plates were not aeriually contaminated. As a result, in the definitive experiment reported here, only two fungal colonies appeared, both on the same control plate, in 138 plates of which 18 were controls.

(iv) Sterilisation of sea water

Sea water used for the dilutions had been collected from each sampling site. It was sterilised by autoclaving before use, and its sterility checked by plating onto the three media. All the 18 plates used showed no growth, except for one which contained two fungal colonies (see above).

2. Water content of sediment

The water content of the top 1 cm sediment surface of the two types of sediments was measured so that viable counts could be expressed as colony forming units/g dry sediment. Five replicate samples, each between 4-5 g wet weight, were taken from each sediment and weighed. Samples were then dried in an oven at 60°C for 24 hours. Sediment samples were re-weighed and the dry weights were determined. These results are shown in Table 1. This table also shows the means and standard deviations of the wet and dry weights as well as the percentage water contents of both sediments.

3. Sediment collection and treatment

Sediments from Langbank and Ardmore sampling sites was collected and treated as follows.

The top 1 cm of the surface sediment from both sampling sites at mid-tide level were carefully removed and placed into separate

44.
 TABLE 1. Means and standard deviations of wet and dry weights of Langbank and Ardmore sediments.

| Replicate | Langbank sediment | | Ardmore sediment | |
|----------------------|--------------------|--------------------|--------------------|--------------------|
| | Wet wt (g) | Dry wt (g) | Wet. wt (g) | Dry wt (g) |
| 1 | 4.501 | 3.393 | 5.054 | 3.854 |
| 2 | 4.754 | 3.664 | 4.640 | 3.586 |
| 3 | 4.982 | 3.849 | 4.598 | 3.606 |
| 4 | 5.089 | 3.929 | 5.329 | 4.093 |
| 5 | 4.488 | 3.512 | 5.632 | 4.391 |
| Means \pm s.d. | 4.763 \pm 0.2735 | 3.669 \pm 0.2241 | 5.051 \pm 0.4439 | 3.906 \pm 0.3410 |
| Mean % water content | 29.% | | 29.2% | |

containers. Sediments were transferred to an aquarium at 10°C. Both sediments were mixed by hand to ensure homogeneity.

Nine millilitres of sterile sea water was dispensed in each of six sterile universal bottles (sterilised overlying water from each sampling site was used). Exactly one gramme of wet sediment was transferred into the first sterile universal bottle. The dilution of this bottle was taken to be 10⁰. This mixture was then shaken by hand for 5 minutes (Anderson and Meadows, 1965). One point zero ml of the suspension was transferred into the second universal tube with a sterile 1 ml delivering pipette. The suspension was pipetted up and down several times before withdrawing the pipette from the tube. The dilution of this tube was taken to be 10⁻¹. This procedure was repeated to obtain the 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions. A fresh pipette was used for each dilution (Cruickshank et al., 1975; p. 307).

4. Inoculation of plates

Zero point one millilitres of each dilution (10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵) was pipetted onto the surface of each plate, and spread using a sterile glass triangle. Two replicates were made for each dilution. Plates were incubated with the top of the petri dish uppermost for 6 hours to facilitate absorption of inocula. They were then turned upside down. Plates were incubated at 10°C. Bacterial colonies were counted after 3, 5, 7, 10, 14 and 21 days (Appendix I, Tables 10 and 11, pp. 444 and 445).

5. Viable counts

The number of living bacteria per gramme dry sediment (colony forming units (C.F.U.)/g dry sediment) after 21 days of incubation was calculated as follows.

The dry weight of 1 g of wet sediment used in the viable counts dilution was calculated as $1 \times 3.669/4.763 = 0.7703$ g (Langbank sediment) and as $1 \times 3.906/5.051 = 0.7733$ g (Ardmore sediment) (see Table 1, p. 44). The number of bacteria (C.F.U.) per plate (from Appendix I, Table 11, p. 445) was therefore multiplied by the dilution factor and then divided by 0.7703 for Langbank and 0.7733 for Ardmore. This gave C.F.U./g dry sediment. The data for the second experiment are shown in Table 4, p. 82.

(III) Chemical measurements

The materials and methods in this section includes the measurements of the following factors: (1) salinity; (2) pH; (3) Eh; (4) organic carbon.

1. Salinity

Samples of overlying, interstitial, and channel waters from both sampling sites were transferred into plastic bottles, the lids of which were closed tightly. Samples were brought back to the department and the salinities of the three types of water measured using a salinity refractrometer. Four readings were taken for each water sample.

2. pH measurements

pH of the three types of water (overlying, interstitial, channel) and the sediments from both sampling sites were measured with a Beckman 3560 Digital pH Meter.

The pH meter was standardised with a pH.7 buffer. Electrodes were then dried and placed into the water sample. pH of the sediment was measured by placing a small volume of the sediment into a small petri dish. A few drops of distilled water (pH.7)

were added to the sediment to moisten it. The pH of the sediment was then measured as above. pH readings of sediment were taken to 40 cm depth at 5 cm intervals. Readings were taken at three time intervals of 5, 10 and 20 seconds.

3. Eh measurements

The redox potential (Eh) readings were obtained using an E.I.L. portable pH meter (Model 30C) with a platinum electrode (Laboratory Platinum Electrode 0-100°C, E.I.L. 33 1213 400) and a calomel reference electrode (Laboratory Refillable Reference Electrode Calomel -5 to 90°C, 3-4 mm/pin (1 m), E.I.L. 33 1370 210).

(a) Measurements of sediment and water Eh

Eh readings were taken by inserting the electrodes into the sediment or water sample. Readings were taken after allowing a period of about 5-20 seconds for stabilisation. Care was taken to zero the Eh meter before each reading. Electrodes were treated with great care and were rinsed with distilled water and dried with a tissue after each reading. They were stored in distilled water on cotton wool between the measurements.

A correction factor of +250 mV was added to all the observed readings (mV) in order to express the results in relation to the normal hydrogen electrode (ZoBell, 1946).

(b) Standardisation of the Eh electrodes

Eh electrodes must be regularly standardised preferably before each set of readings if these are taken on separate days.

Standardisation of Eh electrodes can be conducted using two types of buffer.

The first buffer is 0.003M potassium ferricyanide + 0.003M potassium ferrocyanide in 0.1M potassium chloride. This buffer has an Eh of +430 mV at 25°C (Graetz, Kenney and Aspiras, 1973; Whitfield, 1969; ZoBell, 1946).

The second buffer is the quinhydrone ($C_{12}H_{10}O_4$) [(1:4) O: $C_6H_4:O.OH.C_6H_4.OH = 218.21$] buffer used by Jones (1966). I used the quinhydrone buffer method to standardise the Eh electrodes. This buffer was prepared as follows.

- (i) Two buffers of pH4 and pH7 were prepared.
- (ii) Both buffers were saturated with quinhydrone, by adding a few crystals and stirring well until an amber colour solution was obtained.
- (iii) The platinum and calomel reference electrodes were placed in the quinhydrone buffer solutions, and the Eh was measured. The temperature was also measured. Typical Eh values obtained from these solutions were +218 mV at pH 4.0 and +46 mV at pH 7.0, both at 25°C. These values agree with Jones (1966, p. 42). He obtained Eh values of +218 mV and +46 mV for pH4 and pH7 buffers respectively at 25°C. Table 2 shows these figures.

4. Organic carbon

The materials and methods in this section is divided into two parts. The first (a) describes the wet oxidation method to measure the organic carbon in the three sediments. The second (b) tests the efficiency of two cleaning methods (ashing and acid-cleaning) on removing organic carbon from the three sediments.

TABLE 2. The measured Eh of the quinhydrone buffer at three different temperatures as obtained by Jones (1966) for the Ag-AgCl reference electrode and the calomel reference electrode. The potential (mV) for the calomel reference electrode used in this study is also shown.

| pH buffer | pH4 | | | pH7 | | |
|---|------|------|------|-----|-----|-----|
| Temperature ($^{\circ}\text{C}$) | 20 | 25 | 30 | 20 | 25 | 30 |
| Potential (mV) for Ag-AgCl reference electrode Jones (1966) | +268 | +263 | +258 | +92 | +86 | +79 |
| Potential (mV) for calomel reference electrode Jones (1966) | +223 | +218 | +213 | +47 | +41 | +34 |
| Potential (mV) for calomel reference electrode used in this study | +218 | | | +46 | | |

(a) Wet oxidation

The determination of organic carbon levels within the sediment was based on the wet oxidation method (Holmes and McIntyre, 1984, p. 62) as follows.

(i) Preparation of reagents

1. Forty-nine point zero four grammes of normal potassium dichromate ($N \cdot K_2Cr_2O_7$) was dissolved in distilled water and diluted to one litre.
2. The sulphuric acid used was about 98% (B.D.H. reagent grade).
3. One point two five grammes of silver sulphate (Ag_2SO_4) was added to every 100 ml of acid (the silver sulphate removes the interferences of chlorides).
4. The phosphoric acid used was about 85%.
5. Zero point five grammes of diphenylamine was dissolved in 20 ml of water, followed by the addition of 100 ml of conc sulphuric acid.
6. N ferrous sulphate was prepared as follows:
 - (i) Two hundred and seventy-eight grammes of reagent grade $FeSO_4 \cdot 7 H_2O$ was dissolved in water, and 15 ml of conc sulphuric acid was added. This solution was diluted to one litre.
 - (ii) Standardisation of ferrous sulphate solution was made by titrating against 10.5 ml potassium dichromate (British Standards Institution, 1975, p. 46).

(ii) Method

1. Ten millilitres of N $K_2Cr_2O_7$ were added to two grammes of the natural sediment (i.e. either Langbank or Ardmore sediments), followed by 20 ml of conc H_2SO_4 . Thirty grammes of Rockware sediment was used, because its organic content was very low.

2. The solution was shaken by hand for one minute, then placed in a boiling water bath for 15 minutes.

3. The mixture was then cooled, and 200 ml of distilled water, 10 ml of phosphoric acid, and 1 ml diphenylamine indicator solution, were added. Before addition of the indicator, the solution was yellow/orange. On addition of the indicator, the solution turned deep blue/black.

4. The solution was then titrated by adding ferrous sulphate from a burette - drop by drop towards the end point - until the colour flashed to green.

5. Zero point five of dichromate was added to the above mixture (to restore an excess of dichromate) which turned blue again.

6. Ferrous sulphate was then added again drop by drop until the last trace of blue colour disappeared.

7. Ten replicates of each sediment sample were taken.

(iii) Calculation

The amount of carbon present in the three sediment types was therefore expressed by the following equation:

$$C = \frac{v_1 - v_2}{W} \times 0.003 \times 1000$$

where v_1 equals the volume of N $K_2Cr_2O_7$ (10.5 ml)

v_2 equals the volume of ferrous sulphate (ml)

W equals the weight of soil taken.

The result of the above equation was then equal to $mg\ C.g^{-1}$ dry weight.

(b) Removing of organic carbon from the three sediments using ashing and acid-cleaning methods

The efficiency of ashing and acid-cleaning were tested as follows.

A known weight from Langbank, Ardmore and Rockware sediments was taken and divided into two parts. One part was ashed and the other was acid-cleaned. The organic carbon of the three sediments was then determined.

(i) Ashed sediments

Sediments were ashed by dry combustion at $600^{\circ}C$ for 6 hours as follows.

Samples of Langbank, Ardmore and Rockware sediments were placed in an oven and dried at $60^{\circ}C$ for 24 hours. Dried sediments were then placed in a desiccator and allowed to cool for one hour. The three sediments were weighed, and then placed in a Gallenkamp furnace. The samples were ashed at $600^{\circ}C$ for 6 hours. Five replicates were used for each sediment type. The loss of weight on ashing was taken to represent the amount of organic carbon (Holme and McIntyre, 1984, p. 62).

(ii) Acid-cleaning

Sediments were acid-cleaned by adding normal K_2CrO_4 followed by conc H_2SO_4 in the ratio 1:2 by volume. The sediment was then

shaken by hand for one minute, boiled in a water bath for 20 minutes, cooled and washed six times with distilled water. Sediments were then dried in an oven at 60°C for 24 hours and kept in a desiccator until required.

IV. Physical measurements

The materials and methods in this section include the following measurements:

- (1) shear strength;
- (2) particle size analysis.

1. Shear strength

Determination of sediment shear strength at both sampling sites (Langbank and Ardmore) were carried out on site using a Pilcon Hand Vane Tester. These measurements were made to a depth of one metre at 5 cm intervals. Peak and residual readings were taken at each depth as follows.

The plastic cover of the instrument was removed and the vane spindle was screwed in position. The instrument was then ready for use and was forced into the sediment to the required depth. The instrument should rotate clockwise. This was done by holding the instrument in one hand and revolving the head in a clockwise direction at a constant speed. When the sample sheared, the pointer remained at the angular movement and the reading was then taken. Shear strength was determined by reading off the chart supplied by the manufacturer. Values were expressed in Kg/cm^2 .

2. Particle size analysis

Particle size analysis were carried out to find the particle distribution of the three sediment types.

Langbank and Ardmore sediments were dried in an oven at 60°C for 24 hours. Rockware sediment was almost dry when received but was spread on a tray for 48 hours to dry it further. The dried sediments were then placed on a sieve shaker (Endicott Test Sieve Shaker, Model E.F.L.), and sieved for 30 minutes (Allen, 1975). The sediment fractions in the different sieves were weighed. The sieve sizes used are shown below.

| Thesis code number | Mesh size (μm) | ϕ scale |
|--------------------|-----------------------------|--------------|
| 1 | < 38 | > +5.0 |
| 2 | 38 to 45 | +5.0 to +4.5 |
| 3 | 45 to 63 | +4.5 to +4.0 |
| 4 | 63 to 90 | +4.0 to +3.5 |
| 5 | 90 to 125 | +3.5 to +3.0 |
| 6 | 125 to 180 | +3.0 to +2.5 |
| 7 | 180 to 250 | +2.5 to +2.0 |
| 8 | 250 to 355 | +2.0 to +1.5 |
| 9 | 355 to 500 | +1.5 to +1.0 |
| 10 | 500 to 710 | +1.0 to +0.5 |
| 11 | 710 to 1000 | +0.5 to 0.0 |
| 12 | 1000 to 1400 | 0.0 to -0.5 |
| 13 | 1400 to 2000 | -0.5 to -1.0 |
| 14 | > 2000 | < -1.0 |

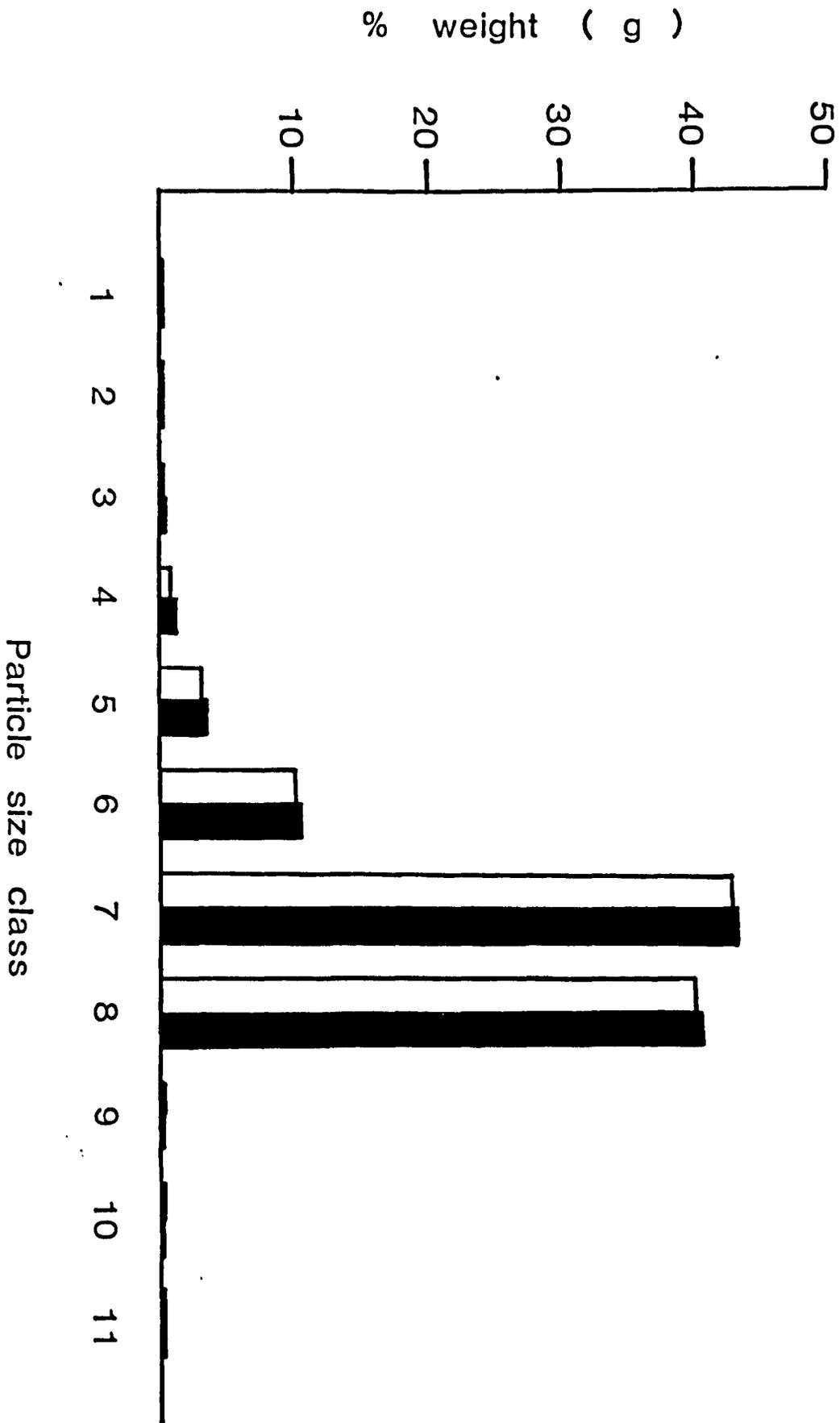
Rockware sediment particles greater than $500 \mu\text{m}$ were sometimes aggregated together. The following experiment was done to determine whether these aggregated particles had any appreciable effect on sediment size distribution. A sample of sediment was divided into two. The aggregated particles from one half were removed, disaggregated, and mixed with the same half again. The other half was not treated, and hence acted as a control. The two halves were sieved separately. The results (Fig. 2) show that there is virtually no difference between the two distributions, and hence the effect is ignored from here onwards.

The graphical techniques used by Inman (1952) was applied to Langbank, Ardmore and Rockware sediments. Using the 95%, 84%, 50%, 16% and 5% intercepts from the y-axis onto the curve, the following parameters were calculated for all the three sediments, Md_{ϕ} ,

$$M_{\phi}, \sigma_{\phi}, \alpha_{\phi}, \alpha_{2\phi} \text{ and } \beta_{\phi}.$$

Figure 2

Rockware sediment. Particle size distribution before (□) and after (■) disaggregation of particles greater than 500 μm .



SECTION I

Results

The Results in this section are divided into four parts. The first (I) gives the animal abundance and animal biomass; the second (II) gives the bacterial counts; the third (III) gives the chemical measurements (salinity, pH, Eh, and organic carbon); the last part (IV) gives the physical measurements (shear strength and particle size analysis).

(I) Animal abundance and animal biomass

The original data for the animal abundance and animal biomass at both sites are shown in Appendix I, Tables 1-4, pp. 435-438. Data are recorded for the number of animals and wet and dry biomass in the top (0-10 cm) and lower (10-20 cm) parts of the three small and the three large cores used at the two sites.

This section of the Results is divided into three parts. The first (1) gives the differences in abundance at the two depths. The second (2) describes the differences in abundance and biomass between small and large cores. The third (3) gives the abundance and biomass of the species.

1. Differences in abundance at the two depths

Inspection of the data in Appendix I, Tables 1-4 shows that most species were only found in the top 10 cm of sediment. Exceptions were N. diversicolor and M. baltica at Langbank, and A. marina at Ardmore which was found at depths greater than 20 cm.

All further statements and statistical analyses refer to the summed data of the 0-10 cm and 10-20 cm.

2. Differences in abundance and biomass between small and large cores

The abundance and biomass of the species on both shores sometimes differed significantly between the small and the large cores. t -tests comparing the small and the large cores are shown in Appendix I, Table 7, p. 441. Inspection of the t -tests shows that the abundances and biomasses were sometimes greater in the small cores and sometimes in the large cores. No particular significance is attached to these differences except that they indicate patchyness in the populations sampled.

3. Differences of abundance and biomass of the species at the two sites

Since there were differences between abundance and biomass between the small and the large cores, statistical analysis of the differences between species was conducted as follows.

The three values for the small cores and the three values for the large cores were tested as six readings, both for the abundance and the biomass data. Comparisons were then conducted between sets of six readings using the non-parametric equivalent of the t -tests - the Mann Whitney U test.

The abundances and biomasses of the species at the two sites are shown in Figures 3, 4, 5 and 6, and in a summary table of percentages (Table 3).

At Langbank, there are only three species which are in order of abundance, C. volutator (85%), N. diversicolor (12.5%) and M. baltica (2.5%) (Fig. 3, Table 3). When expressed as biomass, N. diversicolor is the dominant species (90.6%) followed by C. volutator (7.6%) and M. baltica (1.8%) (Fig. 4, Table 3).

At Ardmore, there are six species compared with the three at Langbank. The most abundant species is P. elegans (92.3%) the other five species are rare in comparison (Fig. 5, Table 3). The biomass data for the six species show that P. elegans and A. marina are the dominant species (44%, 39%) followed by the other species (Fig. 6, Table 3).

Mann Whitney U tests on the abundances and biomasses, comparing pairs of species at the two sites, are shown in Appendix I, Tables 8 and 9, pp. 442, 443 , and in general confirm the above statements.

Figure 3

Langbank. Ranking of species by abundance.

1. Corophium volutator
2. Nereis diversicolor
3. Macoma baltica

Columns represent means. Vertical lines represent standard deviations.

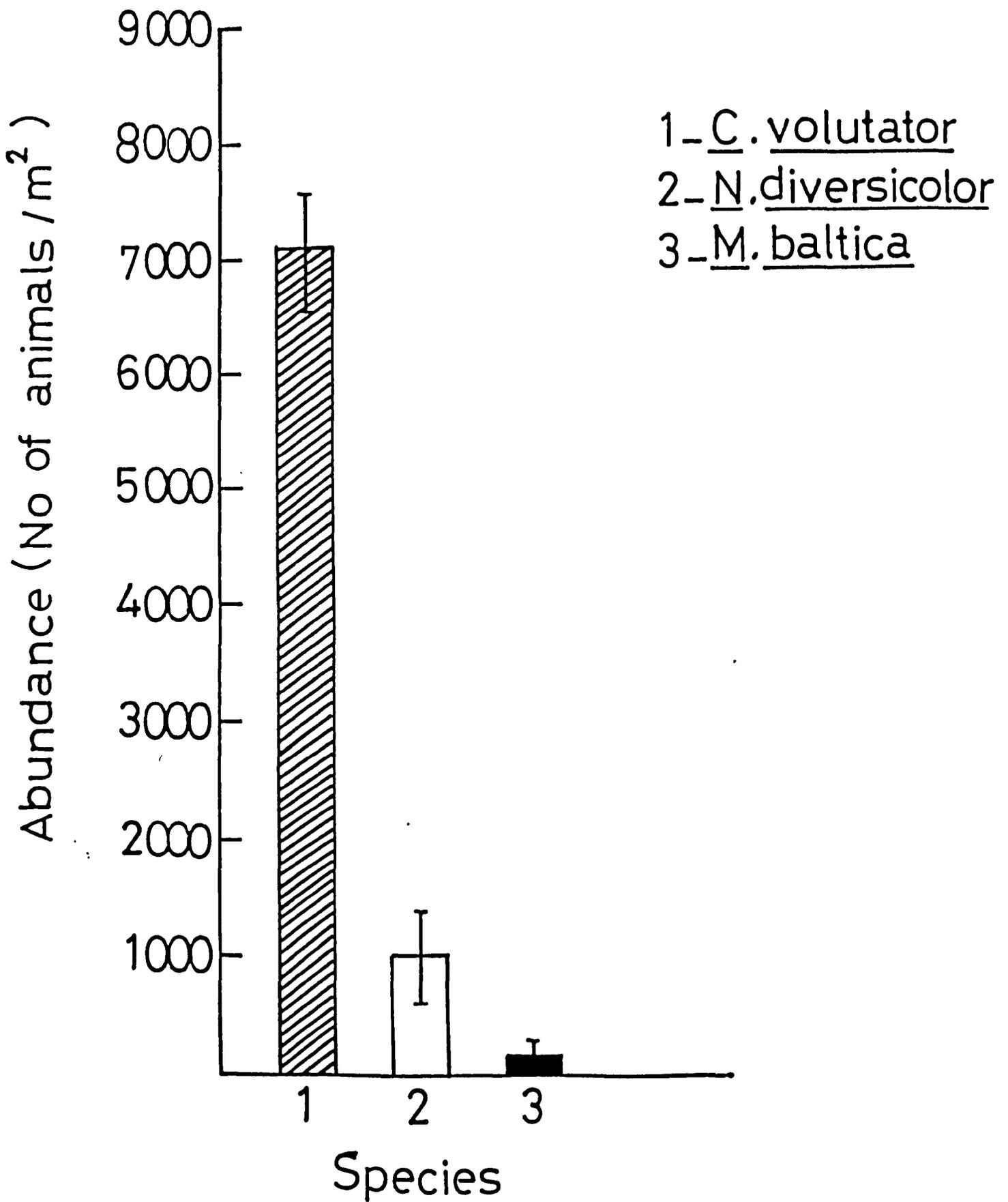


Figure 4

Langbank. Ranking of species by dry biomass (g/m^2).

1. Nereis diversicolor
2. Corophium volutator
3. Macoma baltica

Columns represent means, vertical lines represent standard deviations.

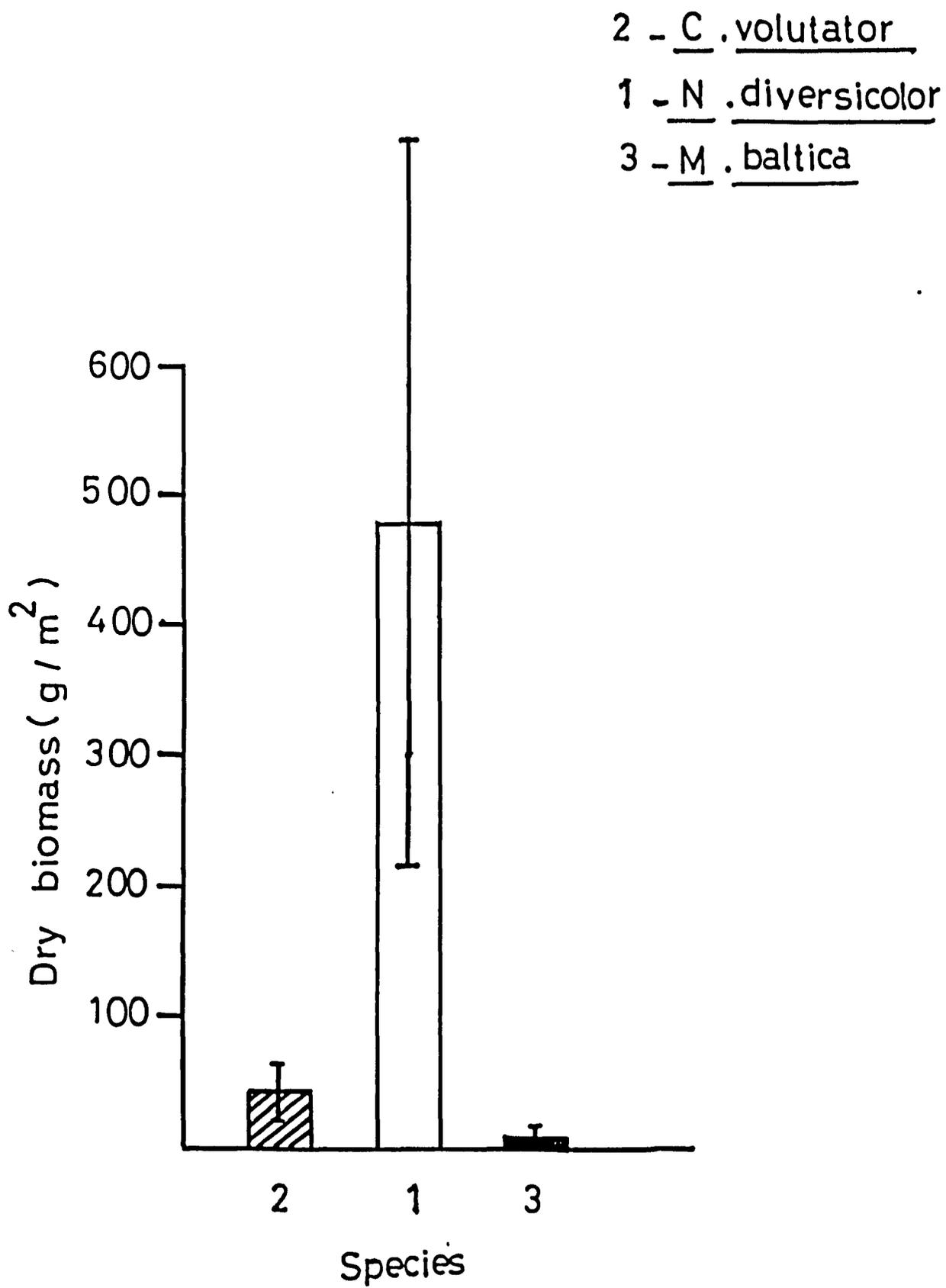


Figure 5

Ardmore Point. Ranking of species by abundance.

1. Pygospio elegans
2. Scoloplos armiger
3. Hydrobia ventrosa
4. Corophium volutator
5. Macoma baltica
6. Arenicola marina

Columns represent means. Vertical lines represent standard deviations.

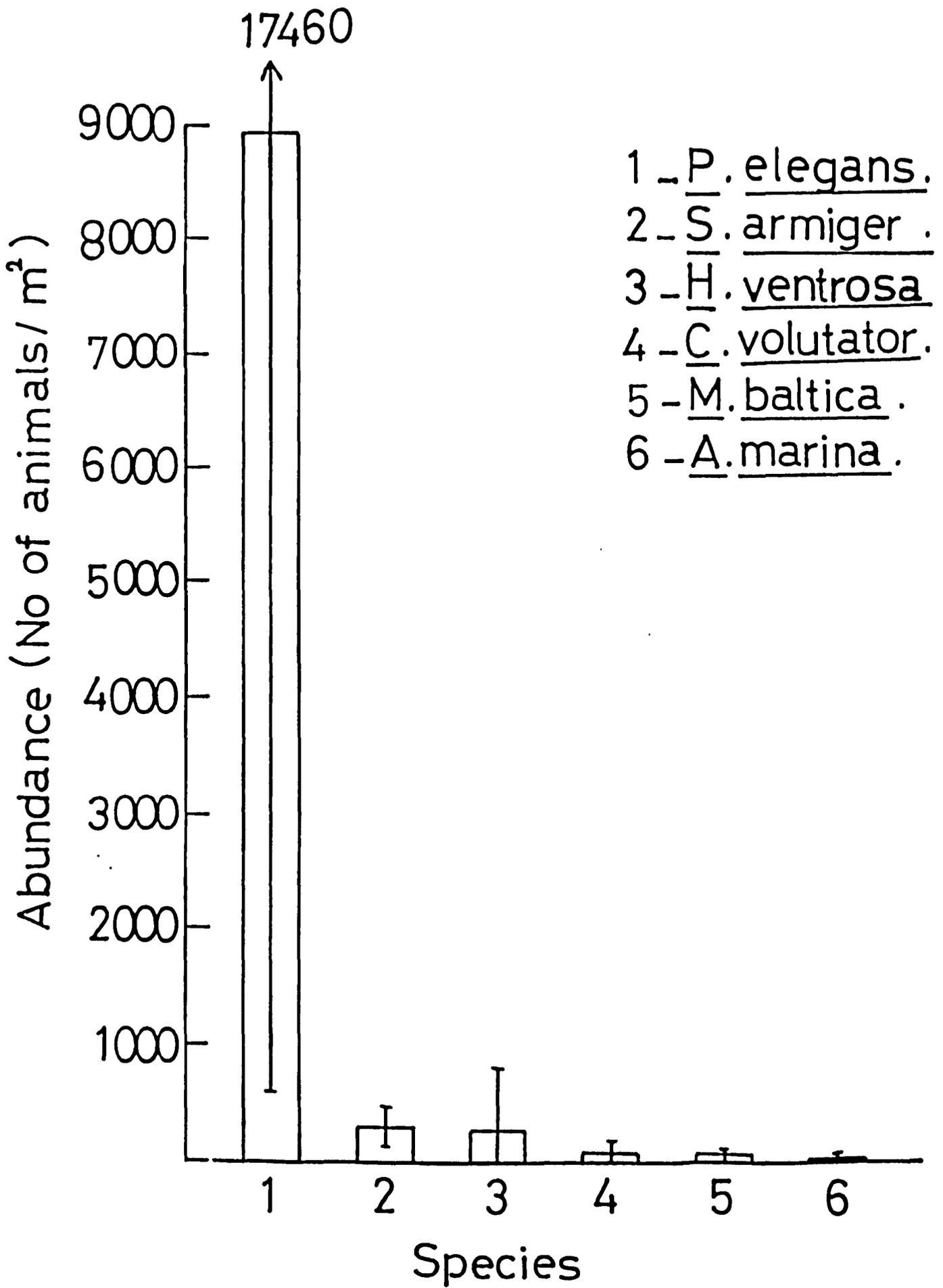


Figure 6

Ardmore Point. Ranking of species by dry biomass
(g/m²).

1. Pygospio elegans
2. Arenicola marina
3. Macoma baltica
4. Scoloplos armiger
5. Hydrobia venturosa
6. Corophium volutator

Columns represent means. Vertical lines represent
standard deviations.

- 1 - P. elegans
- 4 - S. armiger
- 5 - H. ventrosa
- 6 - C. volutator
- 3 - M. baltica
- 2 - A. marina

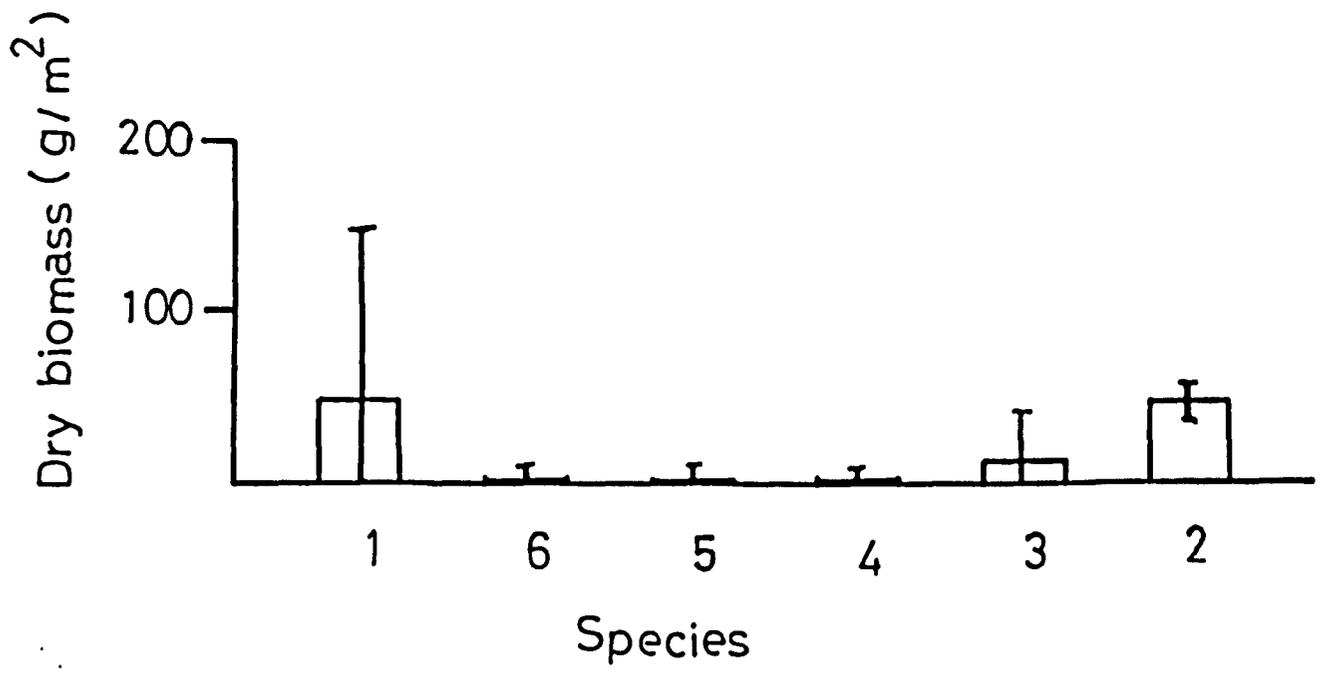


TABLE 3. Percentages of abundances and dry biomasses/m² for the different species at Langbank (LBM) and Ardmore (ARD) sediments.

| Sediment type | Species name | Abundance no./m ² | Dry biomass (g/m ²) |
|---------------|----------------------------|------------------------------|---------------------------------|
| LBM | <u>Corophium volutator</u> | 85% | 7.6% |
| | <u>Nereis diversicolor</u> | 12.5% | 90.6% |
| | <u>Macoma baltica</u> | 2.5% | 1.8% |
| ARD | <u>Pygospio elegans</u> | 92.3% | 44% |
| | <u>Scoloplos armiger</u> | 3.1% | 2.2% |
| | <u>Hydrobia ventrosa</u> | 3.04% | 1.7% |
| | <u>Corophium volutator</u> | 0.66% | 0.34% |
| | <u>Macoma baltica</u> | 0.57% | 13% |
| | <u>Arenicola marina</u> | 0.35% | 39% |

(II) Bacterial counts

Viable counts of bacteria were determined using three types of nutrient media. Samples collected from both Langbank and Ardmore were plated out onto nutrient agar, bacto-marine agar 2216 and teepol-lactose media. Two series of experiments were carried out, the first was preliminary and the second definitive. Results from both experiments were recorded and are presented in Appendix I, Tables 10 and 11, pp. 444, 445 . Results in this section are based on data obtained in the definitive experiment (Appendix I, Table 11, p. 445).

Representative colonies which grew in the first preliminary experiment were photographed and recorded in Plates 3, 4 and 5.

Figures 7, 8 and 9 show the increase in numbers of colonies per plate on the three media with samples obtained from the two sites. These graphs show colony counts reached a maximum after between 7 and 21 days. The 21-day data were therefore used to calculate colony forming units/g dry sediment. They were calculated from those plates having between 30 and 300 colonies, and are shown in Table 4, p. 82.

The data in Table 4 were analysed by a two-way analysis of variance (Appendix I, Table 12, p. 446). This showed a significant first order interaction. One-way analyses of variance were therefore conducted testing differences between media and differences between sites (Table 5, p. 83). These statistical analyses show the following significant differences. Five one-way anovars were conducted. The first two tested the differences between media for each type of sediment. The remaining three tested the differences between sites for each type of media. Results from all these analyses were highly significant ($P < 0.001$).

The following statistically significant conclusions can therefore be drawn from the data in Table 4.

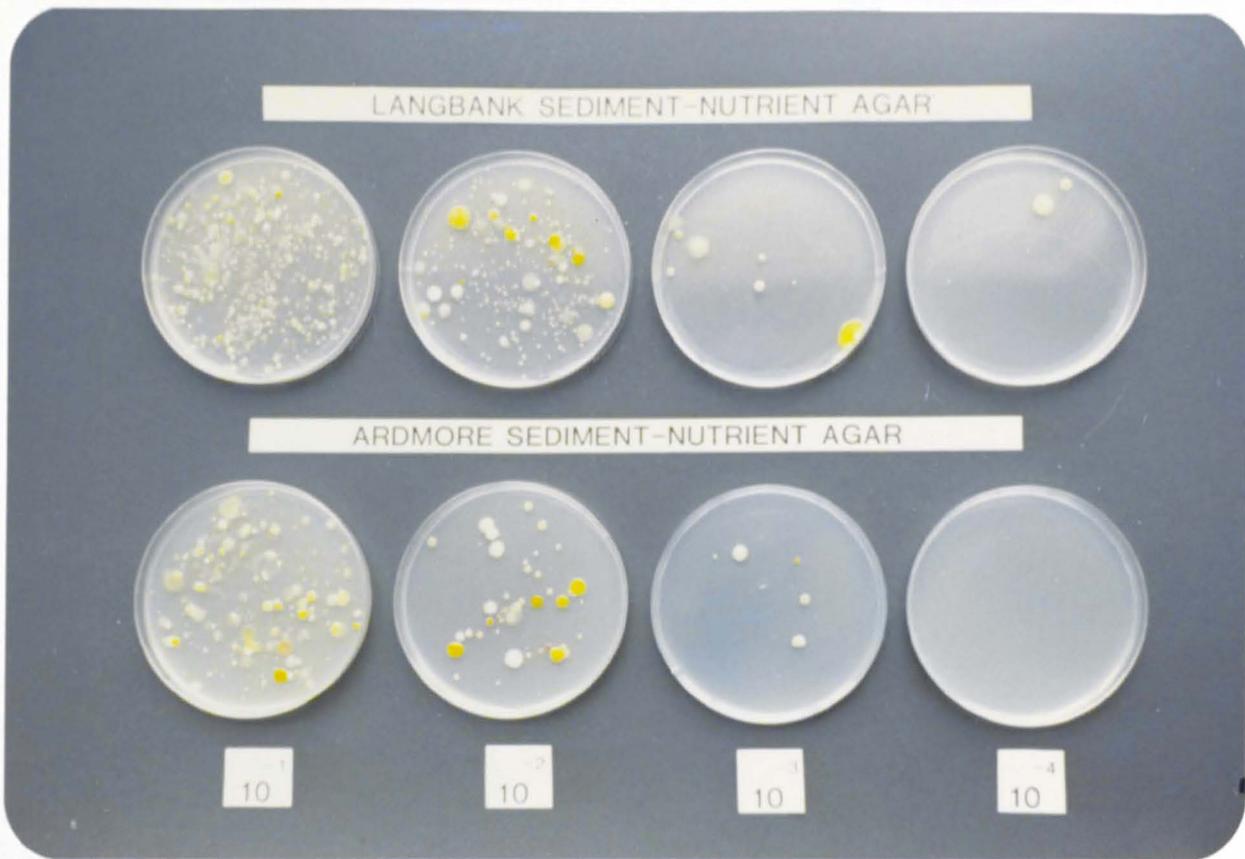
At Langbank and at Ardmore, the highest number of bacteria grew on bacto-marine agar 2216 followed by the nutrient agar (a freshwater medium) and then the teepol-lactose agar. This means that in the sediment at both sites, marine bacteria are most abundant followed by freshwater bacteria and then by coliform bacteria. There were fewer numbers of bacteria recovered from Ardmore sediments on all three media. This means that there were fewer marine and freshwater forms and fewer coliforms at Ardmore than at Langbank. This may be related to a slightly coarser particle size and lower organic content of the sediment at Ardmore compared with Langbank.

Plate 3

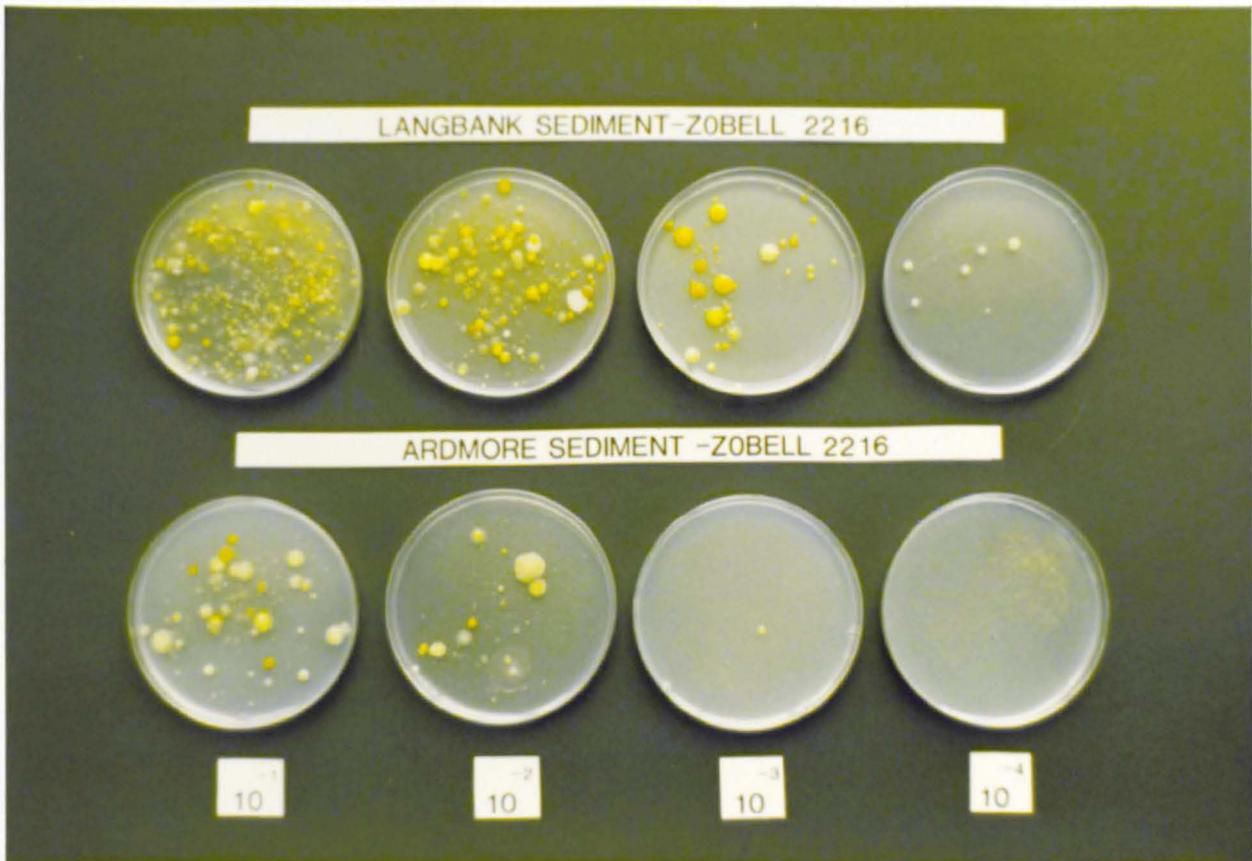
Bacterial colonies grown from Langbank and Ardmore sediments on the nutrient agar plates with four dilutions, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} .

Plate 4

Bacterial colonies grown from Langbank and Ardmore sediments on the bacto-marine agar 2216 medium with four dilutions, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} .



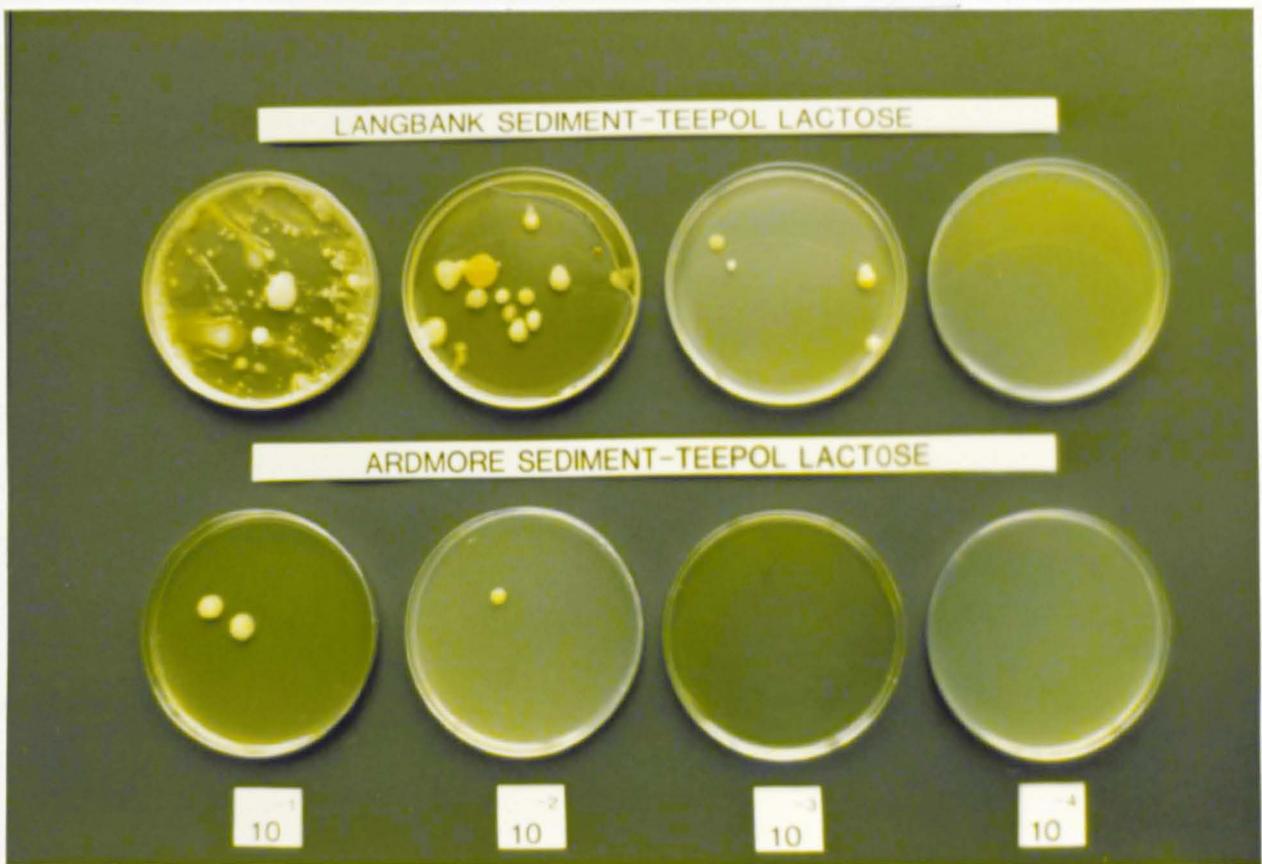
3



4

Plate 5

Bacterial colonies grown from Langbank and Ardmore sediments on the teepol-lactose agar plates with four dilutions, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} .



5

Figure 7

Nutrient agar. Number of colonies per plate over 21 days. Langbank sediment:LBM; Ardmore sediment:ARD. R_1, R_2 = replicate plates. No data are plotted for the 10^{-1} dilution Langbank sediment at 21 days because the numbers of colonies per plate were greater than 1000. No data are plotted for any of 10^{-3} dilution Ardmore sediment plates because the counts were always less than 10/plate.

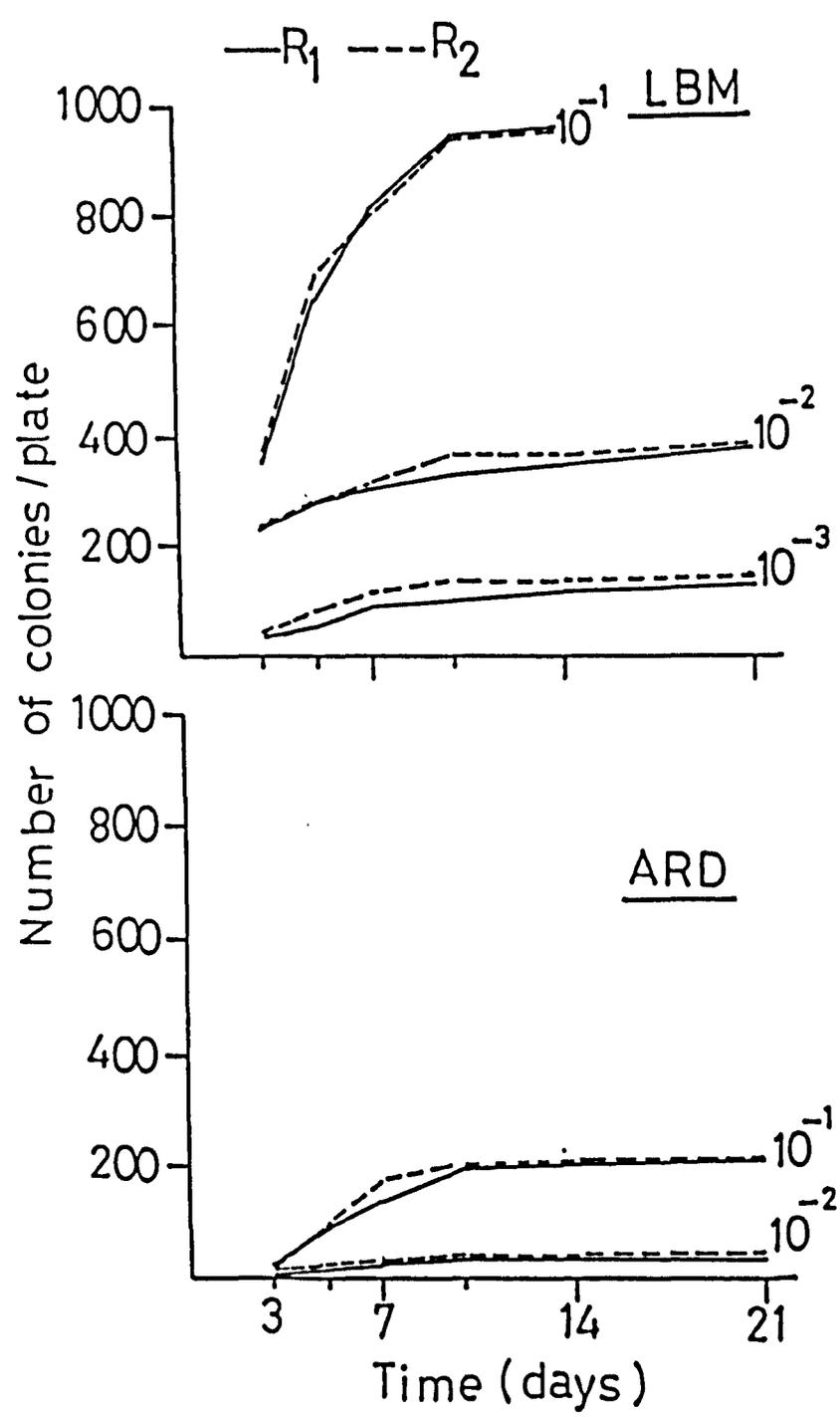


Figure 8

Bacto-marine agar 2216. Number of colonies per plate over 21 days. Langbank sediment:LBM; Ardmore sediment:ARD. R_1, R_2 = replicate plates. No data are plotted for any of the 10^{-1} and 10^{-2} dilutions after 10 days Langbank sediment plates because the number of colonies per plate were greater than 1000.

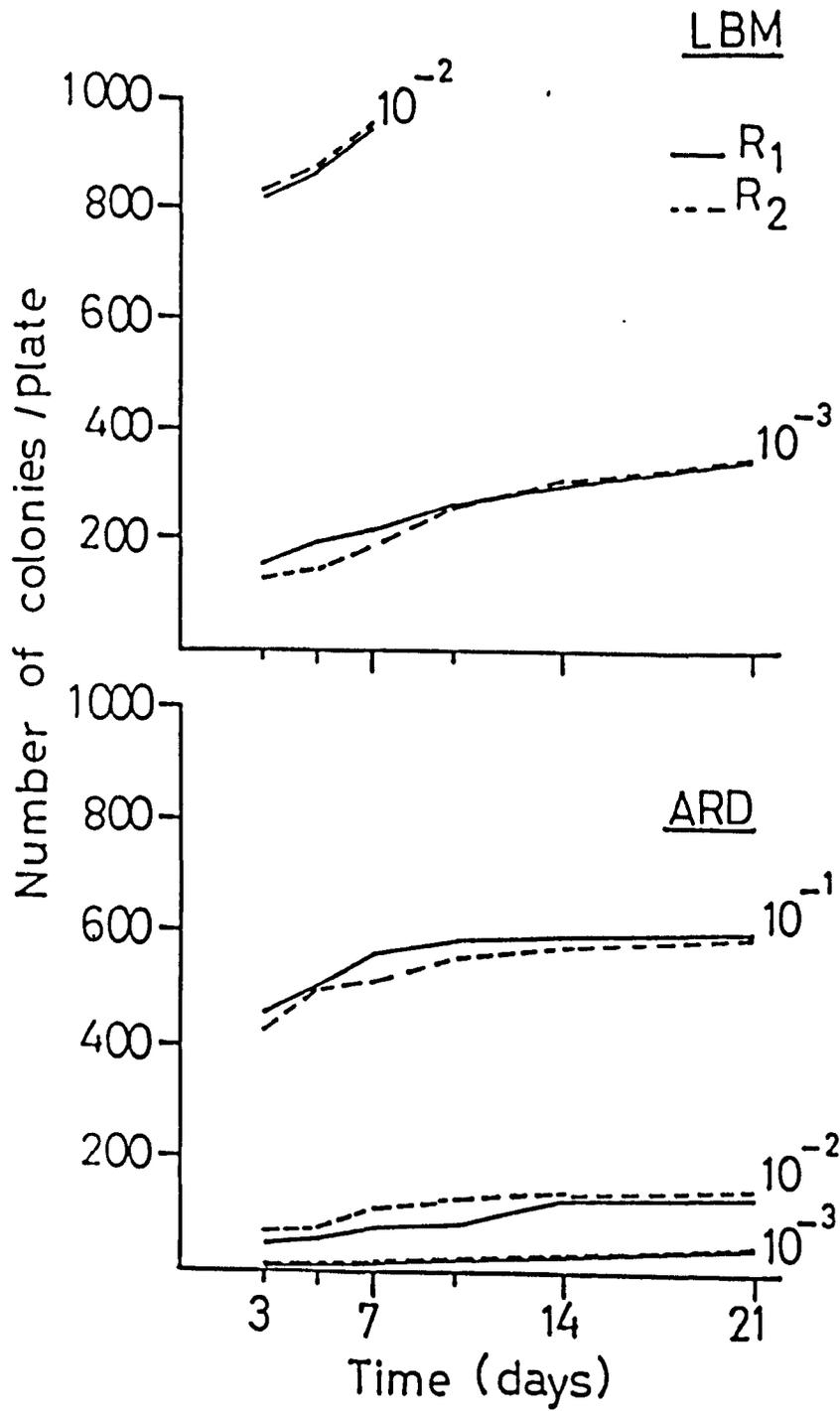


Figure 9

Teepol-lactose agar. Number of colonies per plate over 21 days. Langbank sediment:LBM; Ardmore sediment:ARD. R_1, R_2 = replicate plates. No data are plotted for any of the three dilutions Ardmore sediment plates because the counts were less than 23 colonies for the 10^{-1} , two colonies for the 10^{-2} and no colony with the 10^{-3} .

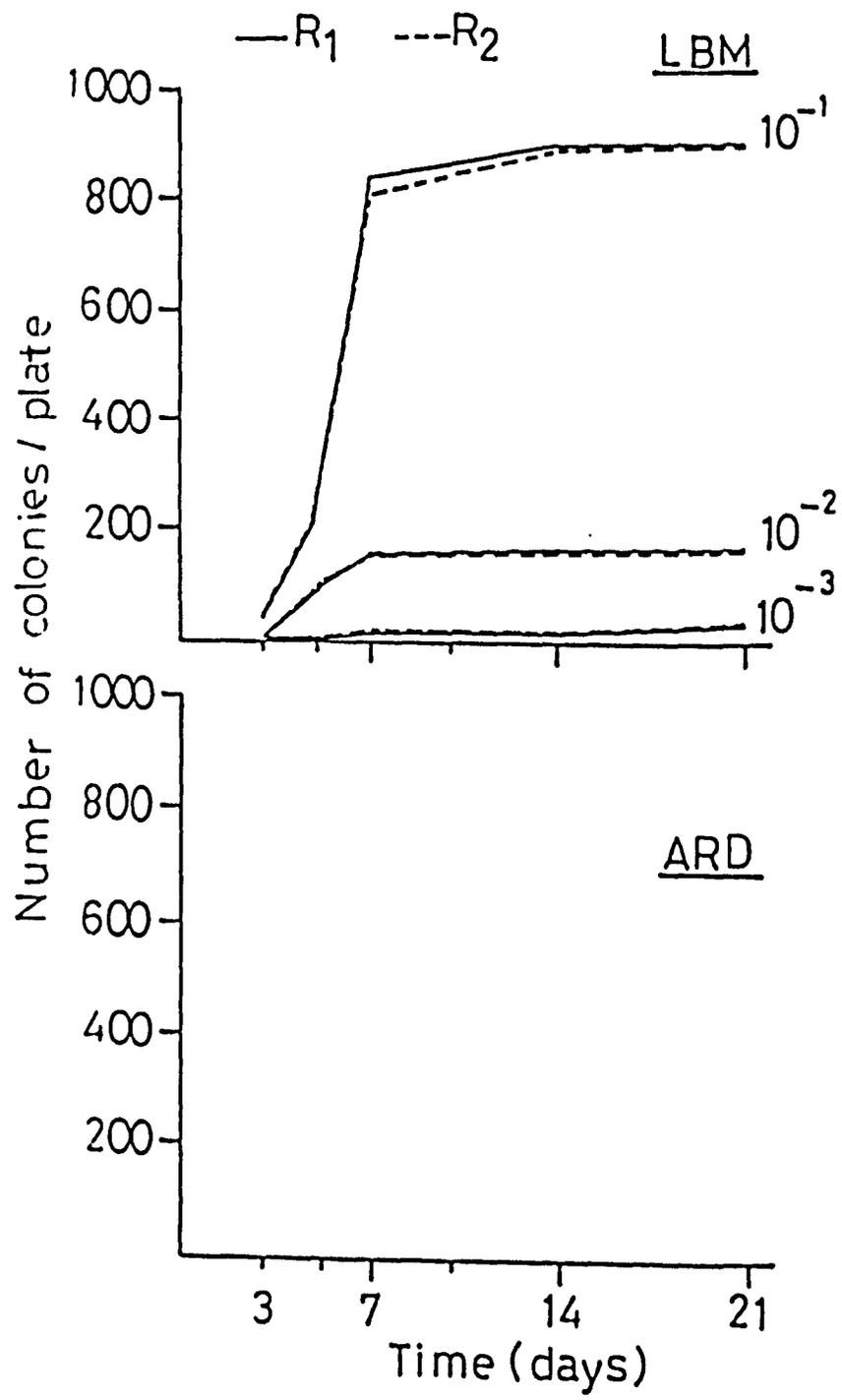


TABLE 4. Number of bacteria per gramme dry weight (c.f.u./g dry sediment) of Langbank (LBM) and Ardmore (ARD) sediments grown on nutrient, bacto-marine 2216, and teepol lactose agar after 21 days. Two replicate plates are presented (R_1 , R_2).

| Sediment type | Replicate | Media | | |
|---------------|-----------|---------------------|------------------------|---------------------|
| | | Nutrient agar | Bacto-marine agar 2216 | Teepol lactose agar |
| LBM | R_1 | 1.675×10^7 | 8.438×10^7 | 2.142×10^6 |
| | R_2 | 1.882×10^7 | 9.087×10^7 | 2.090×10^6 |
| ARD | R_1 | 2.741×10^5 | 1.733×10^6 | 2.845×10^4 |
| | R_2 | 2.793×10^5 | 1.823×10^6 | 2.974×10^4 |

Controls Eighteen replicate plates inoculated with sterile overlying sea water (also used for the dilution series) showed no growth in any case except one replicate with two fungal colonies.

TABLE 5. One-way anovars testing the differences in the number of bacteria recovered from Langbank and Ardmore sediments on three different media (the first two analysis). Differences between both sediments on each medium are also tested (the last three anovars).

| Analysis | Factors | | Sum of squares | Mean squares | D.F. | F-ratio | Probability |
|-----------|------------------------|-------|----------------|--------------|------|---------|------------------------|
| By rows | LBM | Main | 828995.5 | 414497.7 | 2 | 535.9 | P < 0.001 ***** |
| | | Error | 2320.4 | 773.5 | 3 | | |
| | | Total | 831315.9 | | 5 | | |
| | ARD | Main | 358.3 | 179.1 | 2 | 1322.2 | P < 0.001 ***** |
| | | Error | 0.4064 | 0.1355 | 3 | | |
| | | Total | 358.7 | | 5 | | |
| By column | Nutrient agar | Main | 30654.1 | 30654.1 | 1 | 286.2 | P < 0.001 ***** |
| | | Error | 214.2 | 107.1 | 2 | | |
| | | Total | 30868.3 | | 3 | | |
| | Bacto-marine agar 2216 | Main | 736970.7 | 736970.7 | 1 | 699.7 | P < 0.001 ***** |
| | | Error | 2106.4 | 1053.2 | 2 | | |
| | | Total | 739077.2 | | 3 | | |
| | Teepol lactose agar | Main | 435.5 | 435.5 | 1 | 6438.6 | P < 0.001 ***** |
| | | Error | 0.1353 | 0.06764 | 2 | | |
| | | Total | 435.7 | | 3 | | |

(III) Chemical measurements

This part of the Results includes the following factors, salinity, pH, Eh and organic carbon. Organic carbon measurements of Rockware sediment is also included.

1. Salinity

Table 6 shows the salinity for the three types of water at both sampling sites. Salinities of the different types of water at Langbank were lower than their equivalents at Ardmore. Slight variations in salinity were found between the three types of water at Langbank. These values were 26⁰/oo, 24⁰/oo and 27⁰/oo for the overlying, interstitial and the channel water samples respectively. The values for the equivalent types of water at Ardmore were very close and were 31⁰/oo, 30⁰/oo and 31⁰/oo respectively. Ardmore sediment is therefore covered by water of a slightly higher salinity.

2. pH and Eh measurements

This section is divided into three parts: the first (a) gives the pH of the water samples. The second (b) gives the Eh of the water samples. The third (c) gives the pH and Eh of both sediments.

(a) pH of the water samples

Results of the pH measurements of the three types of water are presented in Table 7.

Table 7 shows the means and standard deviations of the pH values of the overlying, interstitial and channel waters. It is clear from the table that Ardmore water samples were slightly more alkaline than the equivalent samples from Langbank, and that the interstitial water at both sites was slightly more acid than the overlying and channel water.

TABLE 6. Means and standard deviations of salinity measure ($^{\circ}/\text{oo}$) of the overlying, interstitial, and channel water at Langbank (LBM) and Ardmore (ARD) sampling areas. Each salinity is the mean of four salinity measurements. (Water temperature = 18°C , room temperature = 20°C .)

| Water type | Salinity ($^{\circ}/\text{oo}$) | |
|--------------------|-----------------------------------|--------------------|
| | LBM | ARD |
| Overlying water | 26.25 ± 0.2887 | 31.25 ± 0.2887 |
| Interstitial water | 24.38 ± 0.2500 | 30.10^* |
| Channel water | 27.05 ± 0.1000 | 31.05 ± 0.1732 |

* All four readings the same. Therefore no standard deviation.

86.
 TABLE 7. Means and standard deviations of pH measure of overlying, interstitial and channel waters at Langbank (LBM) and Ardmore (ARD) sites. Each replicate is the mean of three readings taken at 5, 10 and 20 seconds. (Water temperature = 18°C, room temperature = 20°C.)

| Water type | pH measurements | | | |
|--------------------|-----------------|-----------------|-----------------|-----------------|
| | LBM | | ARD | |
| | R ₁ | R ₂ | R ₁ | R ₂ |
| Overlying water | 8.147 ± 0.04509 | 8.173 ± 0.02082 | 9.500 ± 0.05000 | 9.450 ± 0.05000 |
| Interstitial water | 8.067 ± 0.02887 | 7.983 ± 0.1041 | 8.567 ± 0.3055 | 8.583 ± 0.2021 |
| Channel water | 8.367 ± 0.5204 | 8.083 ± 0.07638 | 9.250 ± 0.05000 | 9.317 ± 0.07638 |

(b) Eh of the water samples

Results of the Eh measurements of the three types of water collected from both sampling sites are shown in Table 8.

This table shows the means and standard deviations of the Eh values of the overlying, interstitial and channel waters. Each figure in Table 8 represents the mean of three readings taken at three time intervals of 5, 10 and 20 seconds.

Variations in Eh values were found between the three types of water at both sampling sites. At Langbank, the Eh values for the overlying, interstitial and channel water samples were 400 mV, 260 mV and 345 mV respectively. At Ardmore, the values for the equivalent water samples were 356.7 mV, 256.7 mV and 360 mV respectively.

In general, the Eh values of the overlying and interstitial water samples at Langbank were slightly higher than the equivalent water samples at Ardmore. The Eh values of the interstitial water samples at the two sites were lower than the Eh values of the overlying and channel waters. This is probably related to the low oxygen content of the interstitial water.

(c) pH and Eh of sediment

Original data of the pH and Eh measurements of sediments at both sites are shown in Appendix I, Tables 13 and 14, pp. 447, 448 respectively.

The means of the sediment pH and Eh are plotted in Figure 10. Standard deviations were not plotted because they were too small. A slight increase with depth in pH values was found in both sediments. These values varied between 7.7-8.4 for Langbank, and 8.04-9.2 for

TABLE 8. Means and standard deviations of Eh values (mV) of the overlying, interstitial, and channel water samples at Langbank (LBM) and Ardmore (ARD) sites. Each figure is the mean of three readings taken at 5, 10 and 20 seconds.

| Water type | Sediment type | |
|--------------------|----------------|----------------|
| | LBM | ARD |
| Overlying water | +400.0* | +356.7 ± 5.774 |
| Interstitial water | +260.0* | +256.7 ± 5.774 |
| Channel water | +345.0 ± 5.000 | +360.6* |

*Standard deviation equal to zero

Ardmore sediments. Ardmore sediment had slightly higher pH values than Langbank sediment at all depths. In addition, Ardmore sediment had a slightly wider pH range (1.16) than Langbank (0.7).

The pH values were analysed by nine 1 x 2 one-way anovars compared differences at each depth between Langbank and Ardmore, and by two 1 x 9 one-way anovars comparing differences between the different depths at each site. In these anovars the two readings at each of the three times were treated as one cell containing six replicates. The results of these anovars are shown in Tables 9 and 10. All of these are significant, and substantiate the above conclusions.

The Eh trends (Fig. 10) were similar in both sediments. They were high at the surface, fell to a minimum at 5 cm and then rose again to remain approximately constant between 10 and 40 cm, with a slight peak at 20 cm.

The Eh values were analysed by nine 1 x 2 one-way anovars compared differences at each depth between the Langbank and Ardmore sediments, and by two 1 x 9 one-way anovars comparing differences between the different depths at each site. In these anovars the three readings at each of the three times were treated as one cell containing three replicates. The results of these anovars are shown in Tables 11 and 12. The results from the first nine one-way anovars (Table 11) were significant in 5/9 comparisons. In these five comparisons, the Langbank sediment had a higher Eh at 0 cm, 20 cm and 35 cm, and the Ardmore sediment a higher Eh at 5 cm and 10 cm. This means that the Langbank sediment was slightly more aerobic at the surface and at depths 0, 20 and 35 cm, while the Ardmore sediment was slightly more aerobic in the 5-10 cm range.

Significant differences were also found between the different depths at each site (Table 12). It is clear from Figure 10 that this is mainly due to the low Eh at 5 cm and higher Eh at the surface and at 20 cm.

Figure 10

Means of pH and Eh (mV) measurements of Langbank (LBM ●—●) and Ardmore (ARD ▲---▲) sediments to 40 cm depth, at 5 cm intervals. Maximum standard deviation for the pH values was ± 0.3873 , and the maximum standard deviation for the Eh values was ± 10.00 . These standard deviations are too small to plot.

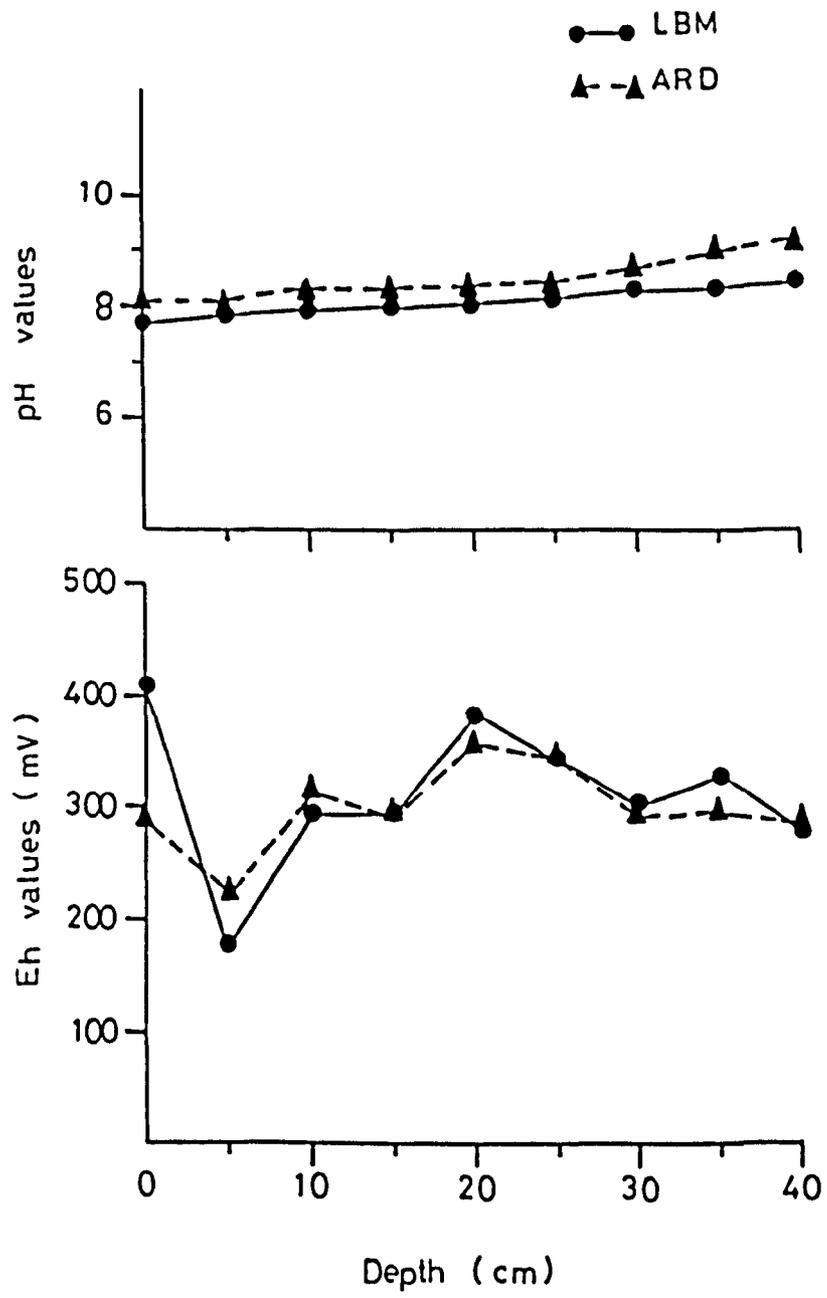


TABLE 9. One-way analyses of variance comparing the pH values of Langbank and Ardmore sediment at nine different depths (cm).

| Depth (cm) | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|------------|---------|----------------|--------------|-----|---------|----------------------------|
| 0 | Main | 0.2730 | 0.2730 | 1 | 36.95 | P < 0.001 ***** |
| | Error | 0.07388 | 0.007388 | 10 | | |
| | Total | 0.3469 | | 11 | | |
| 5 | Main | 0.08333 | 0.08333 | 1 | 6.397 | 0.05 > P > 0.025 * |
| | Error | 0.1303 | 0.01302 | 10 | | |
| | Total | 0.2136 | | 11 | | |
| 10 | Main | 0.2914 | 0.2914 | 1 | 32.49 | P < 0.001 ***** |
| | Error | 0.08968 | 0.008968 | 10 | | |
| | Total | 0.3811 | | 11 | | |
| 15 | Main | 0.2133 | 0.2133 | 1 | 16.33 | 0.005 > P > 0.001 ***** |
| | Error | 0.1306 | 0.01306 | 10 | | |
| | Total | 0.3440 | | 11 | | |
| 20 | Main | 0.1323 | 0.1323 | 1 | 9.657 | 0.01 > P > 0.005 *** |
| | Error | 0.1370 | 0.0137 | 10 | | |
| | Total | 0.2693 | | 11 | | |
| 25 | Main | 0.1323 | 0.1323 | 1 | 12.23 | 0.01 > P > 0.005 *** |
| | Error | 0.1082 | 0.01082 | 10 | | |
| | Total | 0.2405 | | 11 | | |
| 30 | Main | 0.4602 | 0.4602 | 1 | 5.434 | 0.05 > P > 0.025 * |
| | Error | 0.8469 | 0.08469 | 10 | | |
| | Total | 1.307 | | 11 | | |
| 35 | Main | 1.449 | 1.449 | 1 | 107.8 | P < 0.001 ***** |
| | Error | 0.1344 | 0.01344 | 10 | | |
| | Total | 1.583 | | 11 | | |
| 40 | Main | 1.849 | 1.849 | 1 | 87.86 | P < 0.001 ***** |
| | Error | 0.2104 | 0.02104 | 10 | | |
| | Total | 2.059 | | 11 | | |

TABLE 10. One-way analysis of variance comparing pH values measured at nine different depths at 5 cm intervals at Langbank (LBM) and Ardmore (ARD) sediments.

| Sediment | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|----------|---------|----------------|--------------|-----|---------|------------------------|
| LBM | Main | 2.019 | 0.2524 | 8 | 18.11 | P < 0.001 ***** |
| | Error | 0.6274 | 0.0139 | 45 | | |
| | Total | 2.647 | | 53 | | |
| ARD | Main | 7.402 | 0.9252 | 8 | 33.74 | P < 0.001 ***** |
| | Error | 1.234 | 0.02742 | 45 | | |
| | Total | 8.636 | | 53 | | |

TABLE 11. One-way analysis of variance comparing the Eh values of Langbank and Ardmore sediments at nine different depths at 5 cm intervals.

| Depth (cm) | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|------------|---------|----------------|--------------|-----|---------|---------------------------|
| 0 | Main | 21600.0 | 21600.0 | 1 | 432.00 | P < 0.001 ***** |
| | Error | 200.0 | 50.00 | 4 | | |
| | Total | 21800.0 | | 5 | | |
| 5 | Main | 3266.7 | 3266.7 | 1 | 196.0 | P < 0.001 ***** |
| | Error | 66.70 | 16.70 | 4 | | |
| | Total | 3333.3 | | 5 | | |
| 10 | Main | 816.7 | 816.7 | 1 | 49.00 | P < 0.001 ***** |
| | Error | 66.70 | 16.70 | 4 | | |
| | Total | 883.3 | | 5 | | |
| 15 | Main | 66.7 | 66.70 | 1 | 1.000 | 0.50 > P > 0.25 |
| | Error | 266.7 | 66.70 | 4 | | |
| | Total | 333.3 | | 5 | | |
| 20 | Main | 816.7 | 816.7 | 1 | 24.50 | 0.005 > P > 0.001 **** |
| | Error | 133.3 | 33.30 | 4 | | |
| | Total | 950.0 | | 5 | | |
| 25 | Main | 0.000 | 0.000 | 1 | 0.000 | P > 0.75 |
| | Error | 133.3 | 33.30 | 4 | | |
| | Total | 133.3 | | 5 | | |
| 30 | Main | 150.0 | 150.0 | 1 | 4.500 | 0.10 > P > 0.05 |
| | Error | 133.3 | 33.30 | 4 | | |
| | Total | 283.3 | | 5 | | |
| 35 | Main | 1666.7 | 1666.7 | 1 | 100.0 | P < 0.001 ***** |
| | Error | 66.7 | 16.7 | 4 | | |
| | Total | 1733.3 | | 5 | | |
| 40 | Main | 66.70 | 66.70 | 1 | 1.000 | 0.50 > P > 0.25 |
| | Error | 266.7 | 66.70 | 4 | | |
| | Total | 333.3 | | 5 | | |

TABLE 12. One-way analysis of variance comparing the Eh values at nine different depths at Langbank (LBM) and Ardmore (ARD) sediments.

| Sediment | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|----------|---------|----------------|--------------|-----|---------|--------------------|
| LBM | Main | 106051.8 | 13256.5 | 8 | 397.7 | P < 0.001 ***** |
| | Error | 600.0 | 33.30 | 18 | | |
| | Total | 106651.8 | | 26 | | |
| ARD | Main | 36429.6 | 4553.7 | 8 | 111.8 | P < 0.001 ***** |
| | Error | 733.3 | 40.70 | 18 | | |
| | Total | 37163.0 | | 26 | | |

3. Organic carbon

The results of the organic carbon before and after ashing and acid-cleaning for the three sediment types are shown in Table 13. The statistical analyses of these results are given in Table 14 and Appendix I, Table 15.

The organic carbon values with the three sediment types were first analysed by a two-way analysis of variance. Factor A in this anovar was the three sediment types, Langbank, Ardmore and Rockware sediments. Factor B was the three different treatments, control (before cleaning), ashing and acid-cleaning (Appendix I, Table 15). The interaction factor in this anovar was highly significant ($P < 0.001$). Therefore, breakdown statistical analyses were conducted using a series of student t-tests (Table 14).

The following conclusions can be drawn from the data in Table 13 and the t-tests in Table 14.

The percentage reductions of the organic carbon after ashing and after acid-cleaning were 83%, 80% in Langbank, 81%, 79% in Ardmore and 55%, 39% in Rockware sediments respectively. This, of course, reflects the fact that Rockware sediment is a much cleaner sediment.

It is interesting to note that although the percentage losses of organic carbon after ashing and acid-cleaning are much higher in Langbank and Ardmore sediments than Rockware sediment, all the absolute values in mg C_g^{-1} sediment are still significantly higher in Langbank and Ardmore than Rockware (Table 14, t-tests 1, 3, 4, 6, 7, 9). For example, there is a loss in organic carbon after ashing for Langbank, Ardmore and Rockware sediments of 83%, 81% and 54.5% respectively, but the absolute value for the three mentioned sediments

TABLE 13. Langbank, Ardmore and Rockware sediments. Organic carbon before treatment (control), after ashing and after acid-cleaning (mean \pm s.d. mg c g⁻¹ dry sediment). n = number of observations.

| Treatment | Langbank sediment | | | Ardmore sediment | | | Rockware sediment | | |
|---------------------|-------------------|----|---|------------------|----|---|-------------------|----|---|
| | % loss | n | Means \pm s.d. (mg c g ⁻¹) | % loss | n | Means \pm s.d. (mg c g ⁻¹) | % loss | n | Means \pm s.d. (mg c g ⁻¹) |
| Before treatment | - | 10 | 6.161 \pm 0.07430 | - | 10 | 5.312 \pm 0.2508 | - | 10 | 0.06492 \pm 0.005076 |
| After ashing | 83.2% | 10 | 1.033 \pm 0.1601 | 81.24% | 10 | 0.9965 \pm 0.1474 | 54.5% | 10 | 0.02951 \pm 0.009274 |
| After acid-cleaning | 80.4% | 10 | 1.208 \pm 0.2616 | 79.29% | 10 | 1.100 \pm 0.07937 | 38.8% | 10 | 0.03974 \pm 0.01969 |

TABLE 14. Students t-tests analysis of the organic carbon of Langbank (LBM), Ardmore (ARD) and Rockware (RWS) sediments before and after ashing and acid-cleaning.

| Data compared | D.F. | Students t | Probability |
|--|------|------------|----------------------|
| <u>Comparison between Langbank, Ardmore and Rockware sediments</u> | | | |
| <u>Before treatments</u> | | | |
| 1. LBM and RWS | 9 | 258.8 | P < 0.001 ***** |
| 2. ARD and LBM | 9 | 9.541 | P < 0.001 ***** |
| 3. ARD and RWS | 9 | 2096.6 | P < 0.001 ***** |
| <u>After ashing</u> | | | |
| 4. LBM and RWS | 9 | 19.79 | P < 0.001 ***** |
| 5. ARD and LBM | 9 | 0.7091 | 0.5 > P > 0.4 |
| 6. ARD and RWS | 9 | 217.4 | P < 0.001 ***** |
| <u>After acid-cleaning</u> | | | |
| 7. LBM and RWS | 9 | 14.08 | P < 0.001 ***** |
| 8. ARD and LBM | 9 | 0.07698 | P > 0.9 |
| 9. ARD and RWS | 9 | 154.2 | P < 0.001 ***** |
| <u>Langbank sediment</u> | | | |
| 10. LBM before/after ashing | 18 | 91.88 | P < 0.001 ***** |
| 11. LBM before/after acid-cleaning | 18 | 57.59 | P < 0.001 ***** |
| 12. LBM after treatments ashing/ acid-cleaning | 18 | 2.800 | 0.02 > P > 0.01 ** |
| <u>Ardmore sediment</u> | | | |
| 13. ARD before/after ashing | 9 | 2.893 | 0.02 > P > 0.01 ** |
| 14. ARD before/after acid-cleaning | 9 | 9.983 | P < 0.001 ***** |
| 15. ARD after treatments ashing/ acid-cleaning | 9 | 3.450 | 0.01 > P > 0.005 *** |
| <u>Rockware sediment</u> | | | |
| 16. RWS before/after ashing | 18 | 10.59 | P < 0.001 ***** |
| 17. RWS before/after acid-cleaning | 18 | 3.917 | 0.01 > P > 0.005 *** |
| 18. RWS after treatments ashing/ acid-cleaning | 18 | 1.486 | 0.2 > P > 0.1 |

are 1.033 ± 0.1601 , 0.9965 ± 0.1474 and 0.02951 ± 0.009274 .

Inspection of Table 13 shows that ashing appears to remove more organic carbon than does acid-cleaning. This difference was statistically significant for Langbank and Ardmore sediments (Table 14, t-tests 12, 15) and not significant for Rockware sediment (Table 14, t-test 18).

(IV) Physical measurements

This part contains the in situ shear strength measurements on Langbank and Ardmore sediments and the particle size analysis of Langbank, Ardmore and Rockware sediments.

1. Shear strength

The results of the in situ peak and residual shear strength measurements of Langbank and Ardmore sediments are given in Figures 11 and 12. The original data are shown in Appendix I, Table 16, p. 450.

Shear strength values of Langbank sediment increased progressively with depth (Figure 11). With Ardmore sediment, shear strength increased to a depth of 45 cm, then started to decrease (Figure 12). The highest shear strength value with Ardmore sediment was 0.350 kg/cm^2 at a depth of 45 cm. The highest shear strength value recorded for Langbank sediment was 0.308 kg/cm^2 at 100 cm depth.

The residual shear strength values (kg/cm^2) were lower than the peak values for both Langbank and Ardmore sediment at depths greater than 10 cm and 20 cm respectively. At depths less than 10 cm and 20 cm, peak and residual values were essentially the same.

Figure 11

Shear strength values (kg/cm^2) of Langbank sediment to a depth of 100 cm at 5 cm intervals (P and R are the peak and residual shear strength respectively).

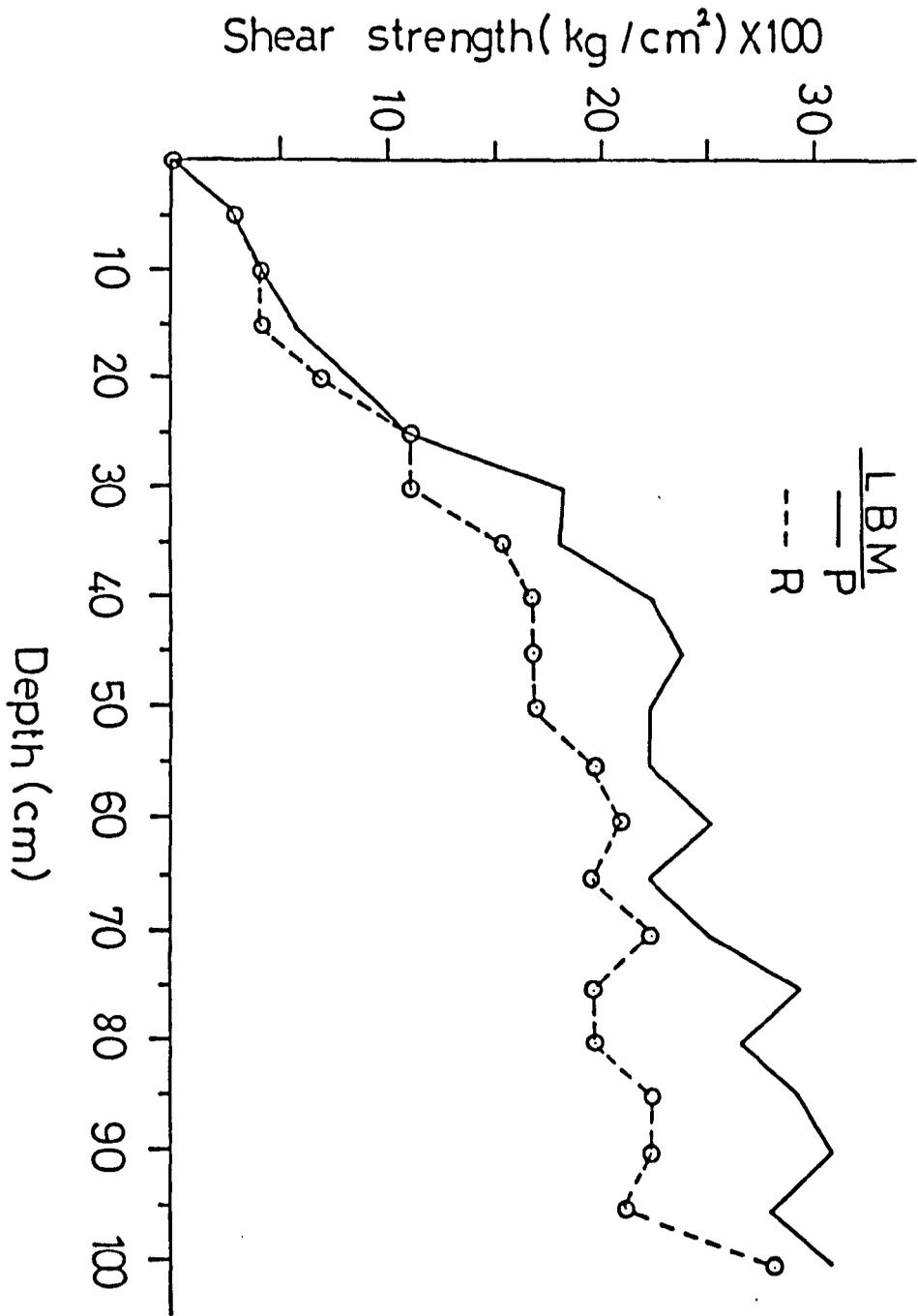
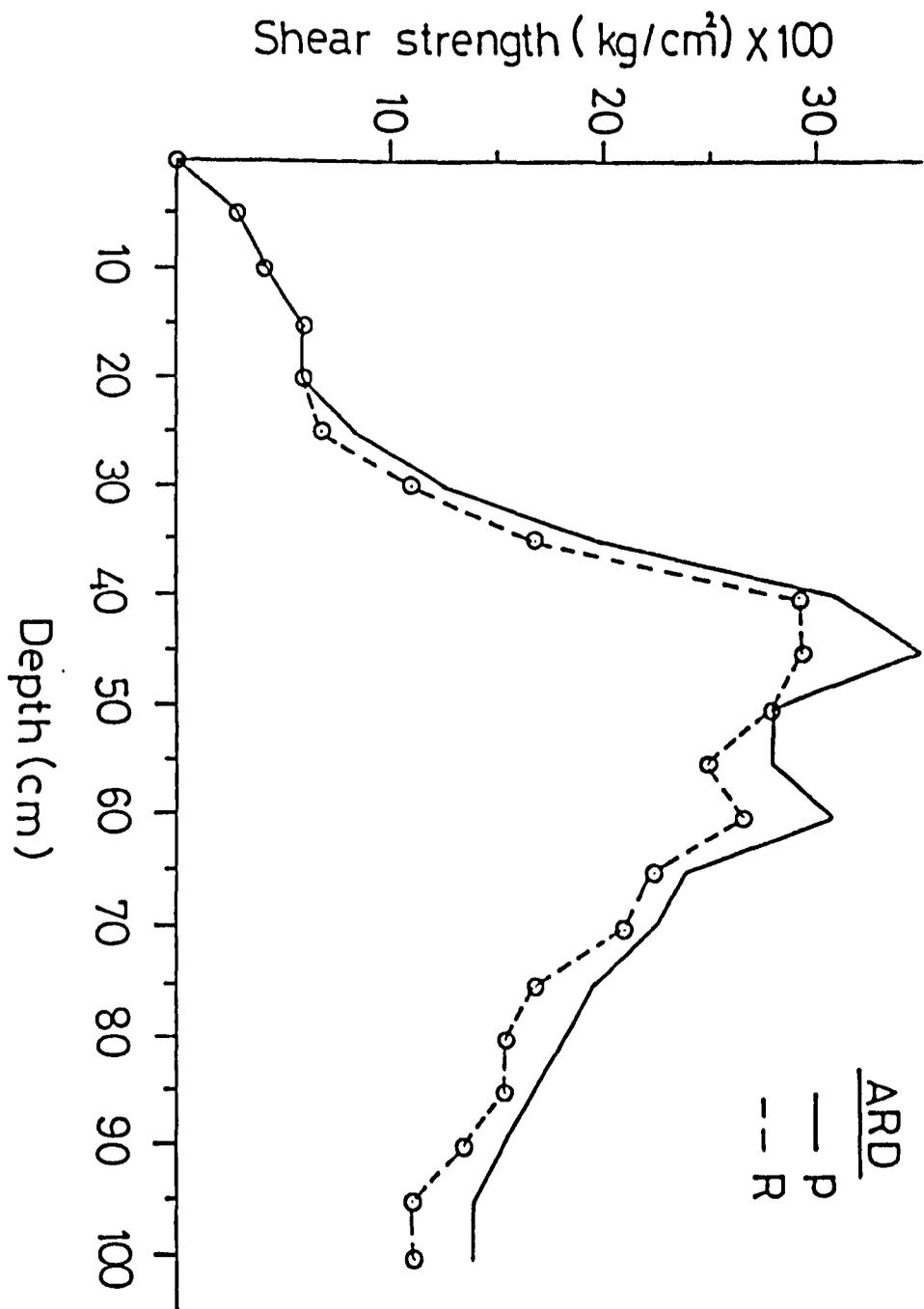


Figure 12

Shear strength values (kg/cm^2) of Ardmore sediment to a depth of 100 cm at 5 cm intervals (P and R are the peak and residual shear strength respectively).



2. Particle size analysis of Langbank, Ardmore and Rockware sediments

This section is divided into three parts. The first (a) describes the ordinary and cumulative size frequency distributions of the three sediments. The second (b) describes Inman's (1952) method for calculating sediment particle size parameters. The third (c) describes the results of applying Inman's method to the three sediments.

(a) Size frequency distribution

The results of this part are presented in Figures 13-18 and Table 15.

Ordinary and cumulative plots of particle size are shown for Langbank in Figures 13 and 14, for Ardmore in Figures 15 and 16 and for Rockware in Figures 17 and 18. Table 15 shows the accepted terminology for the various size fractions, the ϕ scale, the sieves used in this study and the size code used in Figures 13-18.

In general, there was little difference between the size distribution of the three sediments. Most of the size fractions were between 125-710 μm for Langbank (Figures 13 and 14; x-axis 6, 7, 8, 9, 10), and between 90-355 μm for Ardmore sediments (Figures 15 and 16; x-axis 5, 6, 7, 8), and between 125-500 μm for Rockware sediment (Figures 17 and 18; x-axis 6, 7, 8, 9).

(b) Inman's (1952) particle size analysis method

The ϕ scale measures particle size of sediments in terms of $-\text{Log}_2$ (particle diameter in mm) (Table 15).

Figure 19 shows a plot of diameter in ϕ units against cumulative percentage by weight for a hypothetical sediment. It is taken from Inman (1952), who describes the parametric data that can be

Figure 13

| Langbank sediment. | Fractions distribution. | |
|--------------------------------|-------------------------------|--------------------------------|
| 1 = $< 38 \mu\text{m}$; | 2 = 38-45 μm ; | 3 = 45-63 μm ; |
| 4 = 63-90 μm ; | 5 = 90-125 μm ; | 6 = 125-180 μm ; |
| 7 = 180-250 μm ; | 8 = 250-335 μm ; | 9 = 335-500 μm ; |
| 10 = 500-710 μm ; | 11 = 710-1000 μm ; | 12 = 1000-1400 μm ; |
| 13 = 1400-2000 μm ; | 14 = $> 2000 \mu\text{m}$. | |

Figure 14

Langbank sediment. Per cent cumulative plot. 1-14 on the x-axis are the same size fractions as in Figure 13 .

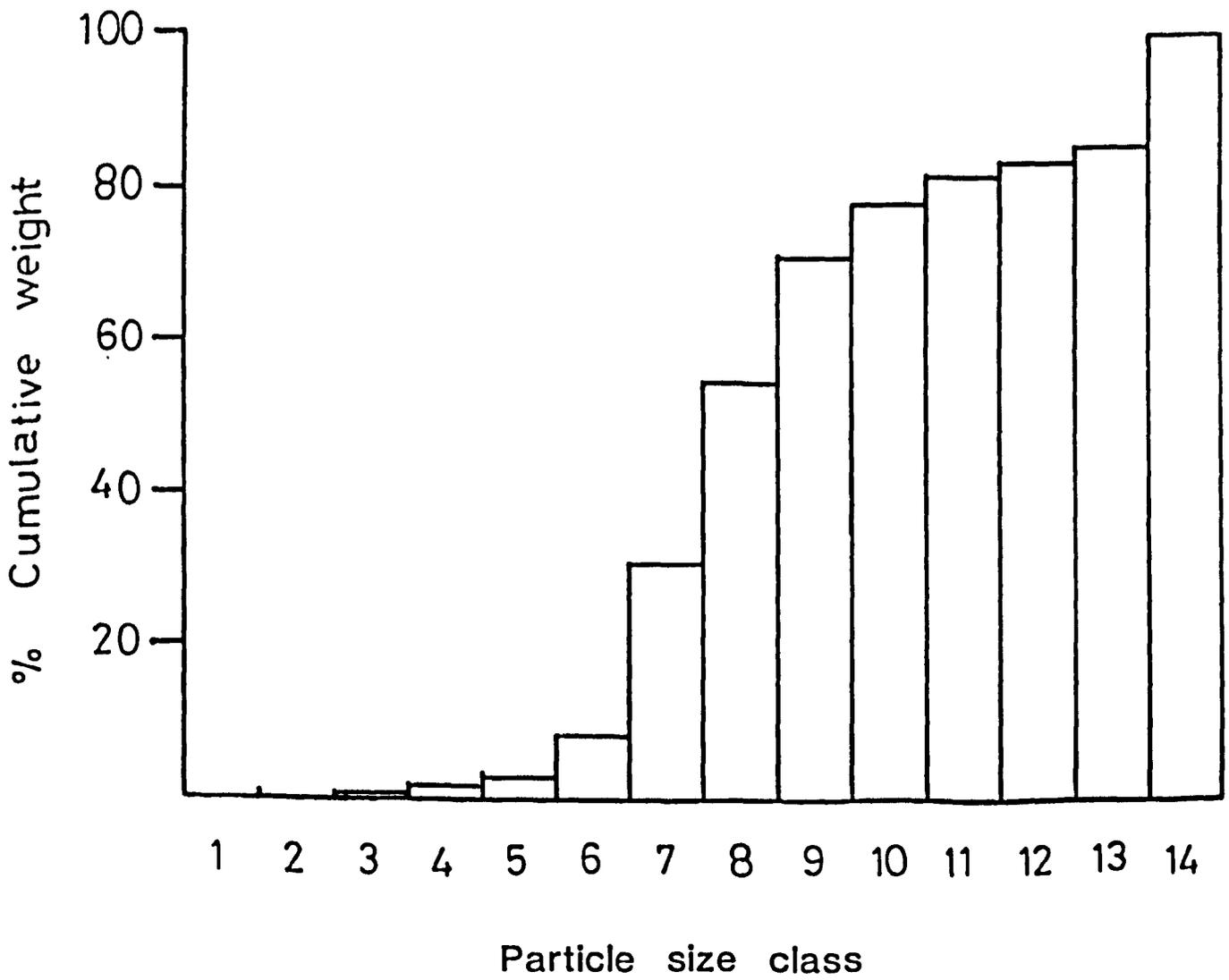
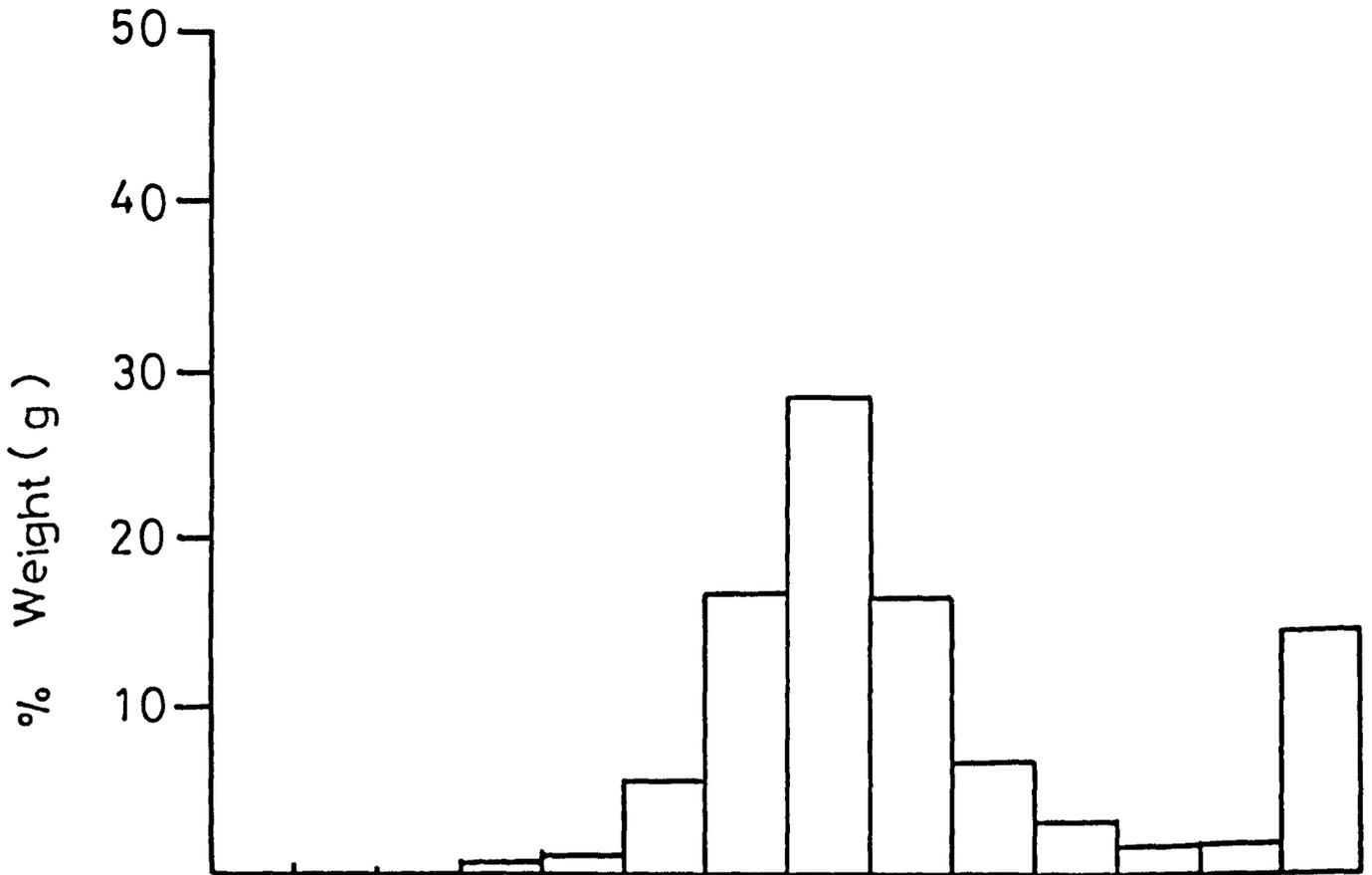


Figure 15

Ardmore sediment. Fractions distribution.

| | |
|-----------------------------|--------------------------------|
| 1 = $< 38 \mu\text{m}$ | 8 = 250-335 μm ; |
| 2 = 38-45 μm ; | 9 = 335-500 μm ; |
| 3 = 45-63 μm ; | 10 = 500-710 μm ; |
| 4 = 63-90 μm ; | 11 = 710-1000 μm ; |
| 5 = 90-125 μm ; | 12 = 1000-1400 μm ; |
| 6 = 125-180 μm ; | 13 = 1400-2000 μm ; |
| 7 = 180-250 μm ; | 14 = $> 2000 \mu\text{m}$. |

Figure 16

Ardmore sediment. Per cent cumulative plot. 1-14

on the x-axis are the same size fractions as in Figure 15.

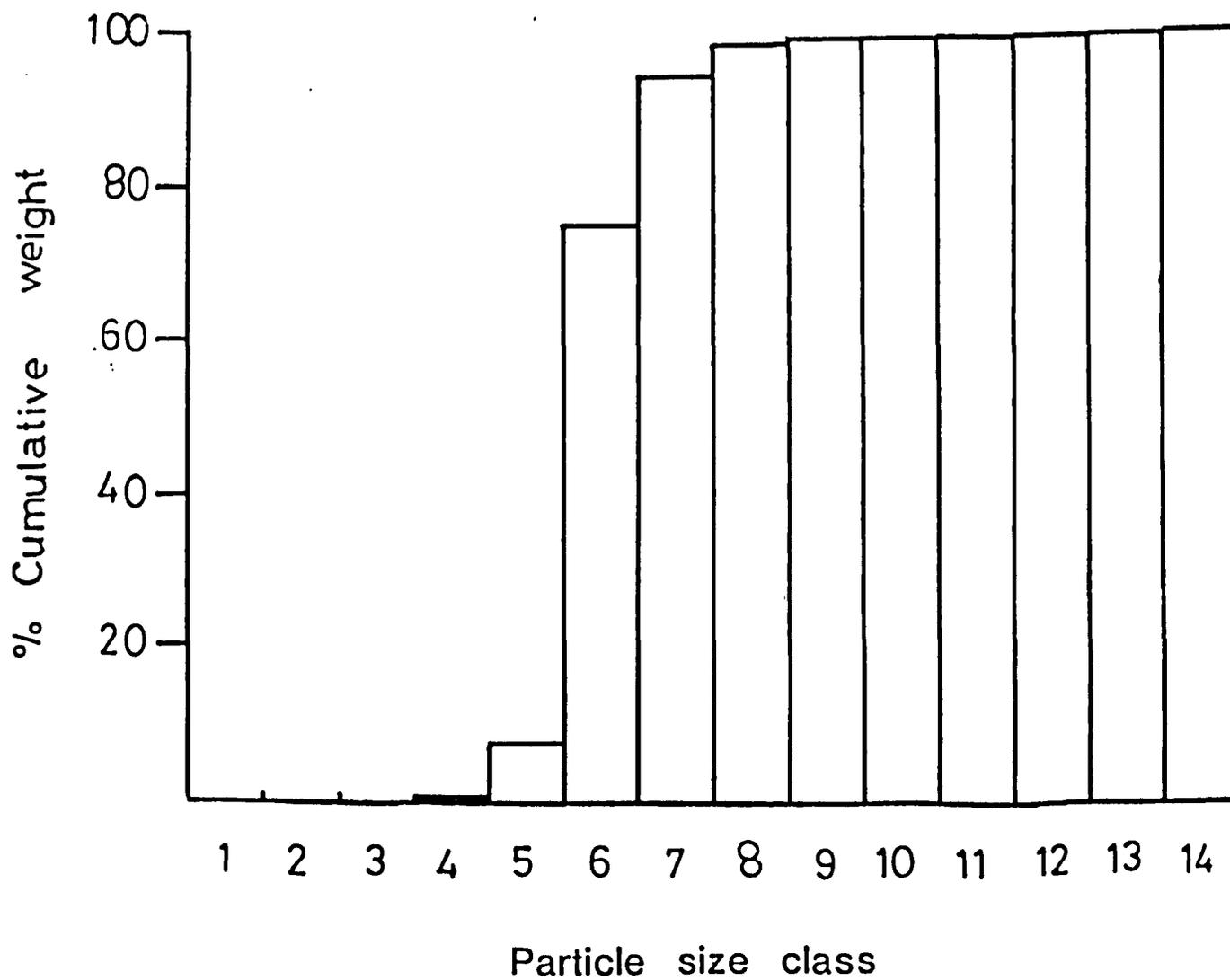
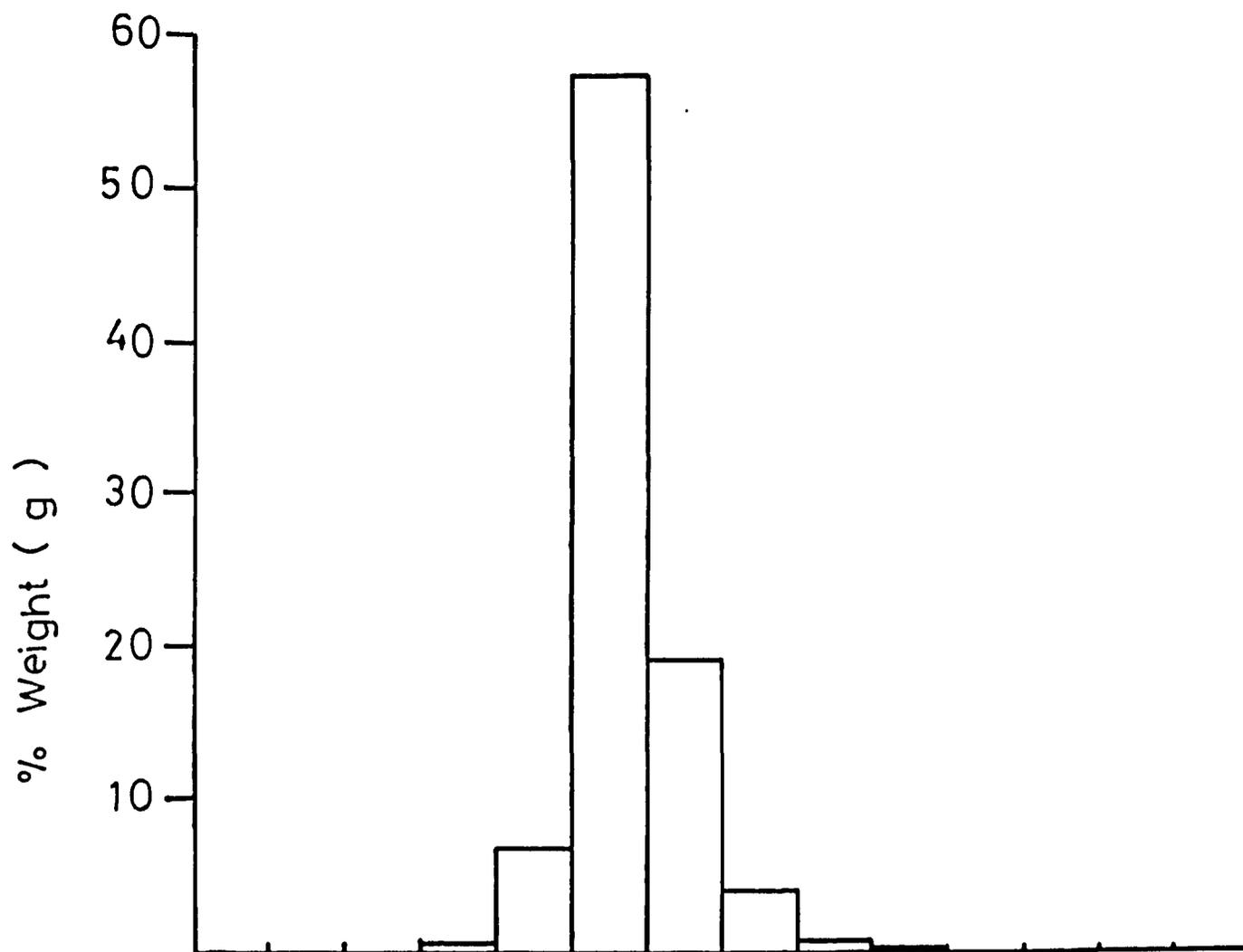


Figure 17

| Rockware sediment. Fractions distribution. | |
|--|--------------------------------|
| 1 = $< 38 \mu\text{m}$; | 8 = 250-335 μm ; |
| 2 = 38-45 μm ; | 9 = 335-500 μm ; |
| 3 = 45-63 μm ; | 10 = 500-710 μm ; |
| 4 = 63-90 μm ; | 11 = 710-1000 μm ; |
| 5 = 90-125 μm ; | 12 = 1000-1400 μm ; |
| 6 = 125-180 μm ; | 13 = 1400-2000 μm ; |
| 7 = 180-250 μm ; | 14 = $> 2000 \mu\text{m}$. |

Figure 18

Rockware sediment. Per cent cumulative plot. Figures 1-14 on the x-axis are the same size fractions as in Figure 17.

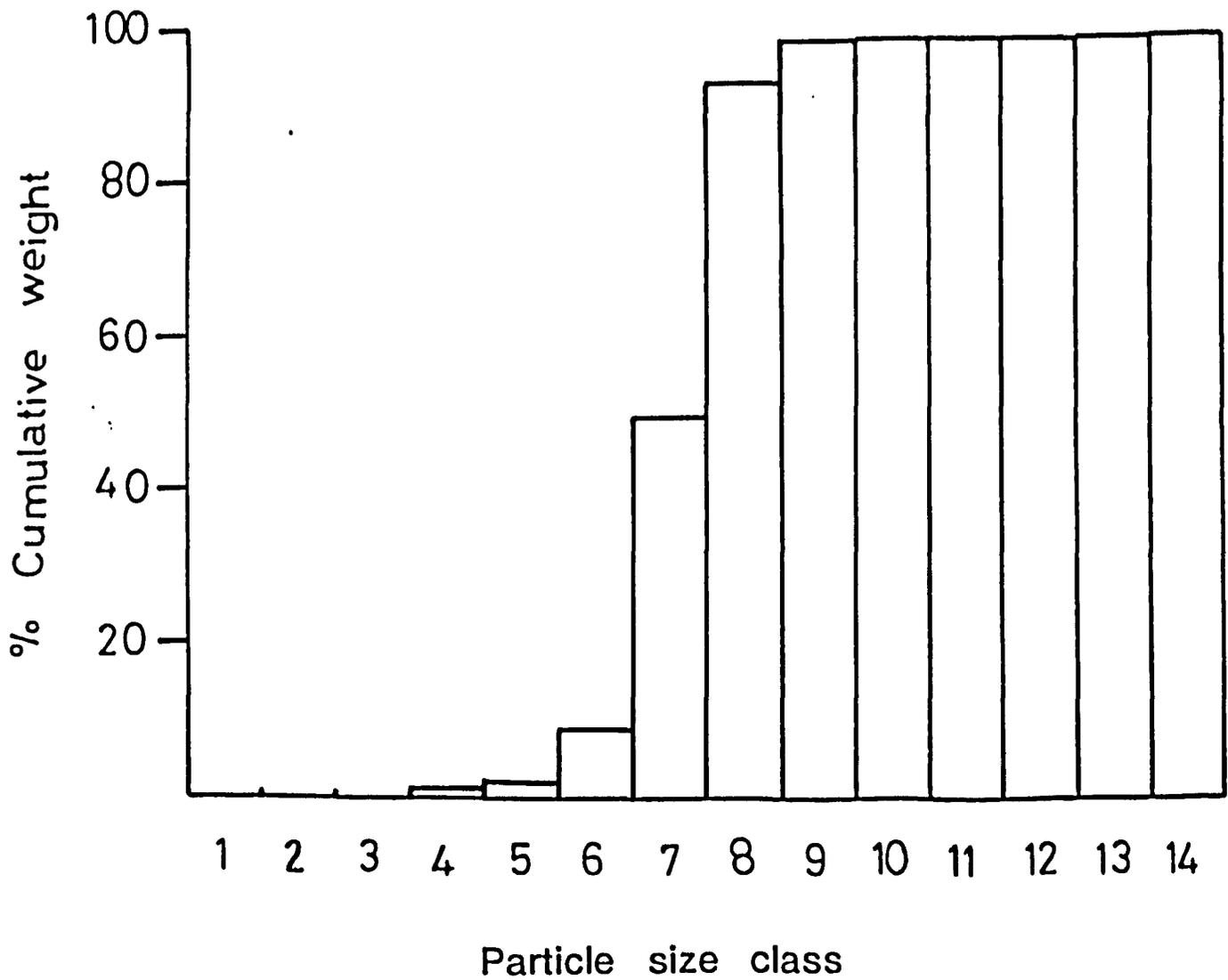
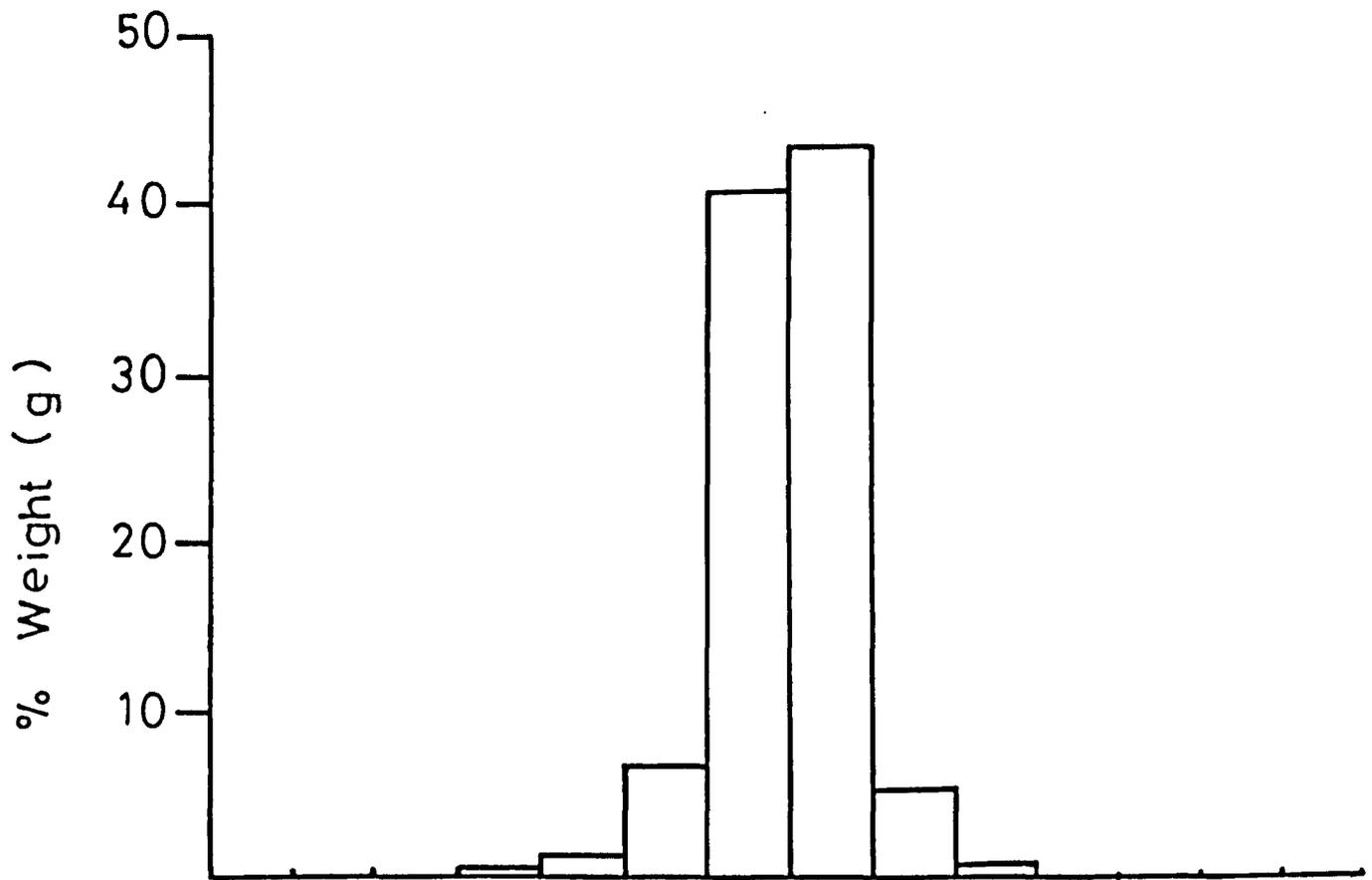


TABLE 15. Classification of sediment by grain size (modified from Buchanan and Kain, 1971).

0 = $-\log_2$ (particle diameter in mm)

1 = $62 \mu\text{m}$ (sand)

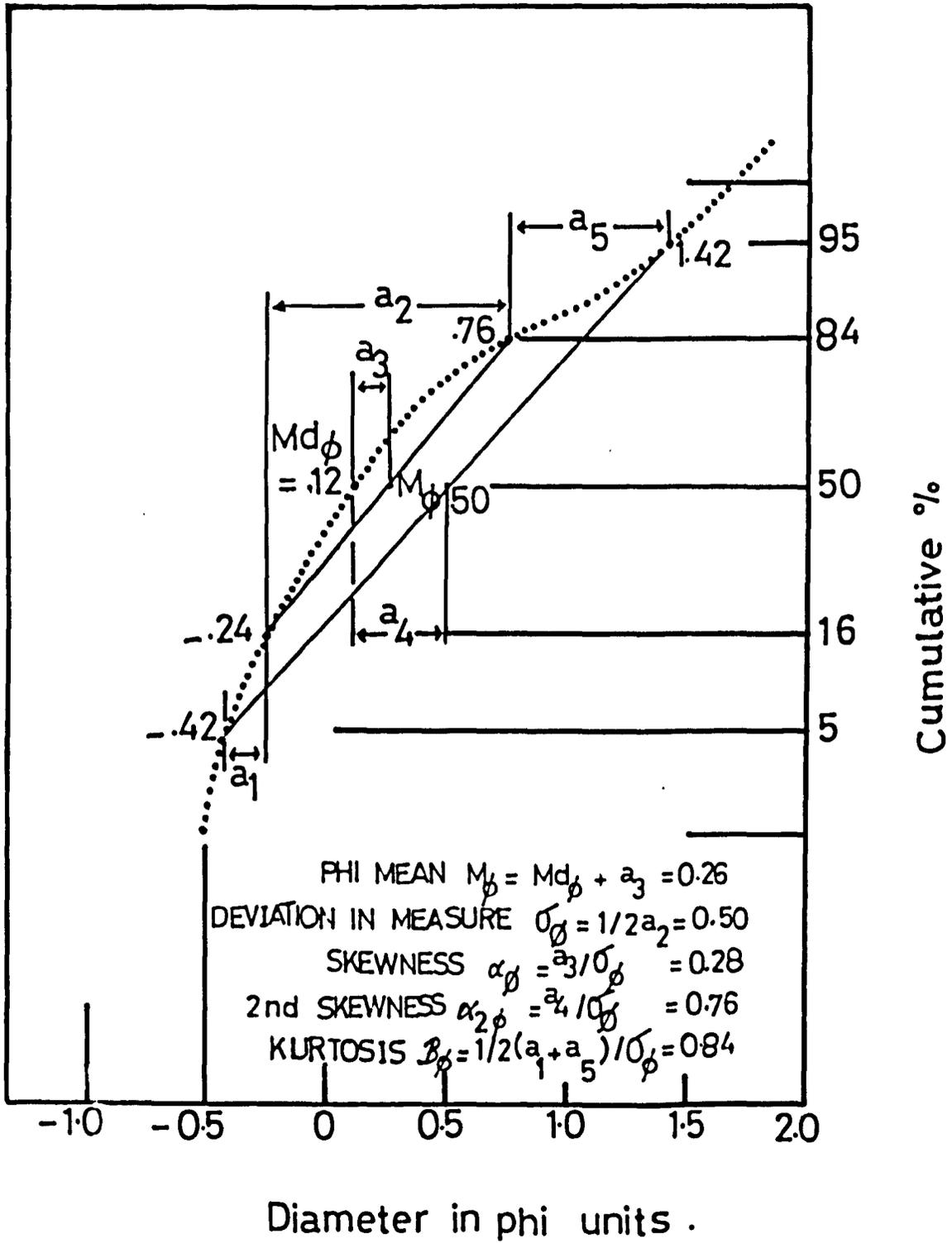
2 = $62 \mu\text{m}$ (silt)

3 = $3.9 \mu\text{m}$ (clay)

| Fractions | | Particle diameter in mm | ϕ | Sieve sizes used (μm) | Code in Figs 13-18 |
|-----------|------------------|-------------------------|--------|------------------------------------|--------------------|
| 1 Sand | very coarse sand | 2 | -1 | >2000 | 14 |
| | | 1.41 | -0.5 | 2000 | 13 |
| | coarse sand | 1 | 0 | 1410 | 12 |
| | | 0.71 | +0.5 | 1000 | 11 |
| | medium sand | 0.50 | +1.0 | 710 | 10 |
| | | 0.351 | +1.5 | 500 | 9 |
| | | 0.250 | +2.0 | 355 | 8 |
| | | 0.177 | +2.5 | 250 | 7 |
| | fine sand | 0.125 | +3.0 | 180 | 6 |
| | | 0.088 | +3.5 | 125 | 5 |
| | very fine sand | 0.062 | +4.0 | 90 | 4 |
| | | 0.044 | +4.5 | 63 | 3 |
| | | 0.031 | +5.0 | 45 | 2 |
| | | 0.022 | +5.5 | 38 | |
| 2 Silt | silt | 0.0156 | +6.0 | | |
| | | 0.0110 | +6.5 | 38 | 1 |
| | | 0.0078 | +7.0 | | |
| | | 0.0055 | +7.5 | | |
| 3 Clay | clay | 0.0039 | +8.0 | | |
| | | <0.0039 | | | |

Figure 19

Diameter in ϕ units plotted against cumulative % weight
for a hypothetical sediment, according to Inman (1952).



obtained from this type of curve. The following parameters

| | |
|------------------------|------------------|
| ϕ median diameter | Md_{ϕ} |
| ϕ mean diameter | M_{ϕ} |
| deviations measure | σ_{ϕ} |
| skewness | α_{ϕ} |
| second skewness | $\alpha_{2\phi}$ |
| kurtosis | β_{ϕ} |

can be obtained by using 95%, 84%, 50%, 16% and 5% intercepts from the y-axis onto the curve. These parameters are defined and calculated in the following paragraphs.

(i) Phi median diameter and phi mean diameter

Both the phi median diameter Md_{ϕ} and the phi mean diameter M_{ϕ} were obtained from points selected from the cumulative curve. They are defined as follows:

The median is the diameter value of the ordinate that divides the frequency distribution curve into two equal areas. Inman (1952) (p. 133) states that it can be defined as:

$$Md_{\phi} = \phi_{50} = M_{\phi} - (\sigma_{\phi} \alpha_{\phi}).$$

The phi mean diameter is the mean particle size in phi units, and Inman (1952, pp. 133-134) states that it can be taken as the average of the 16th and 84th percentile diameters, as follows:

$$M_{\phi} = 1/2 (\phi_{16} + \phi_{84}) = Md_{\phi} + (\sigma_{\phi} \alpha_{\phi}).$$

The phimedian diameter may be obtained directly from the curve without interpolation. Inman states that the median is less affected by extreme values of skewness because it is closer to the modal (or most commonly occurring) diameter than is the mean. However, I used the mean diameter, since it is the more generally accepted estimate of the average particle size.

(ii) Deviation measure (standard deviation)

The standard deviation was obtained from one half the distance between the 16th and 84th percentile diameters on accumulative frequency curve (Inman, 1952, p. 135):

$$\sigma_{\phi} = 1/2 (\phi_{84} - \phi_{16}).$$

(iii) Skewness measure

Skewness is the extent of departure of the mean from the median. It is defined as follows.

1. The primary skewness measure α_{ϕ} is sensitive to skew properties occurring in the bulk of the grain-size distribution, and is the amount of the departure of the distribution from the normal (Inman, 1952, p. 137).

$$\alpha_{\phi} = \frac{1/2(\phi_{16} + \phi_{84}) - Md_{\phi}}{\sigma_{\phi}} = \frac{M_{\phi} - Md_{\phi}}{\sigma_{\phi}}$$

2. The secondary measure of skewness, called the second phi skewness measure, is based on the 5th and 95th percentile diameters.

The secondary skewness $\alpha_2\phi$ is most sensitive to the distribution within the tails of the sediment and is given as

$$\alpha_2\phi = \frac{1/2(\phi_5 + \phi_{95}) - Md_\phi}{\sigma_\phi}$$

by Inman (1952, p. 137).

(iv) Kurtosis measure

The phi Kurtosis measure (flatness or peakedness of a curve) is a dimensionless measure of the average spread between the percentile diameters $\phi_5, \phi_{16}, \phi_{84}, \phi_{95}$, and it is defined as:

$$\beta_\phi = \frac{1/2(\phi_{16} - \phi_5) + 1/2(\phi_{95} - \phi_{84})}{\sigma_\phi} = \frac{1/2(\phi_{95} - \phi_5) - \sigma_\phi}{\sigma_\phi}$$

$$\beta_\phi = \frac{1/2(a_1 + a_5)}{\sigma_\phi}$$

by Inman (1952), p. 138).

Inman (1952) gives no data on a sediment having a normally distributed particle size. This would give a straight line using Inman's plot. I have analysed this situation in Appendix I, pp. 451, 452. The point of this analysis was to give me some idea of what a normal distribution would look like using Inman's analysis, so that if necessary I could compare it with my own data.

(c) Application of Inman's (1952) method to Langbank, Ardmore and Rockware sediments

Inman's (1952) graphical technique shown in Figure 19 was applied to Langbank, Ardmore and Rockware samples, whose curves are plotted in Figure 20. The parameters described above are shown in Table 16 for the three sediments.

Ardmore sediment had the smallest median and mean diameter followed by Rockware and then that of Langbank. The median diameters were less than the mean diameters (note the ϕ scale numbers increase as the particle size decreases). This means that Ardmore and Rockware sediments are slightly finer sediments than Langbank sediment. Langbank had the greatest standard deviation followed by that of Ardmore and then that of Rockware. This means that Langbank sediment has a wider scatter of particle size than Ardmore and Rockware sediments. Ardmore sediment had the largest skewness, followed by Langbank and then by Rockware, while Langbank sediment had the largest kurtosis followed by Rockware and then Ardmore.

Figure 20

Diameter in phi units and cumulative % weight greater than Langbank (LBM), Ardmore (ARD) and Rockware (RWS) sediments.

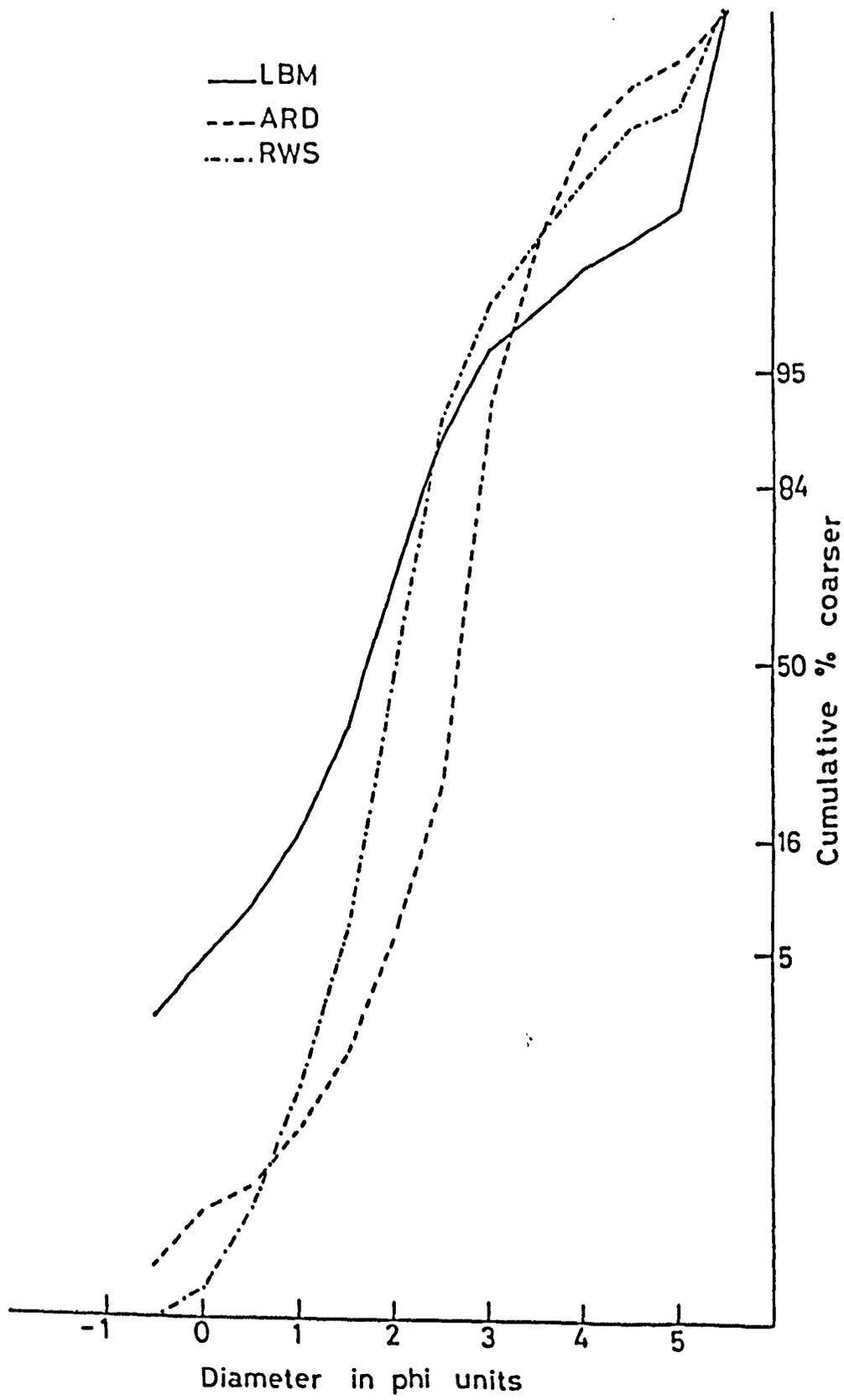


TABLE 16. Langbank (LBM), Ardmore (ARD) and Rockware (RWS) sediments, parameters obtained from Figure 20 using Inman's (1952) graphical method (see Figure 19). Nomenclature and definition from Inman, 1952, Figure 4, Table 1.

| Nomenclature | Definition | Graph data | | |
|---|---|--|--|---|
| | | LBM | ARD | RWS |
| Phi median diameter | $Md_{\phi} = \phi_{50}$ | 1.7 (308 μm) | 2.67 (157 μm) | 2.0 (250 μm) |
| Phi mean diameter | $M_{\phi} = Md_{\phi} + a_3$ | $1.7 + 0.05 = 1.75$ (297.3 μm) | $2.67 + 0.07 = 2.74$ (149.7) | $2 + 0.02 = 2.02$ (246.6) |
| Deviation measure (standard deviation) | $\sigma_{\phi} = 1/2 a_2$ | $1/2(1.31) = 0.65$ (= 637.3 μm) | $1/2(0.96) = 0.48$ (= 717.0 μm) | $1/2(0.65) = 0.325$ (= 798.3 μm) |
| Skewness | $\alpha_{\phi} = a_3 / \sigma_{\phi}$ | $0.05 / 0.65 = 0.07692$ | $0.07 / 0.48 = 0.1458$ | $0.02 / 0.325 = 0.06154$ |
| 2nd skewness | $\alpha_{2\phi} = a_4 / \sigma_{\phi}$ | $0.2 / 0.65 = 0.3076$ | $0.15 / 0.48 = 0.3125$ | $0.08 / 0.325 = 0.2462$ |
| Kurtosis | $\beta_{\phi} = 1/2(a_1 + a_5) / \sigma_{\phi}$ | $1/2(0.9 + 0.55) / 0.65$ = 1.115 | $1/2(0.39 + 0.2) / 0.48$ = 0.6146 | $1/2(0.27 + 0.36) / 0.325$ = 0.9692 |

SECTION I

Discussion

The Discussion in this section is divided into four parts: (I) animal abundance and animal biomass; (II) bacterial counts; (III) chemical measurements; (IV) physical measurements.

(I) Animal abundance and animal biomass

The abundance, biomass, distribution and diversity of a wide range of animal species has been recorded in a number of studies (see Introduction, p. 24 for references). Broadly speaking these studies included animal species belonging to various animal groups - Annelida (Polychaeta), Arthropoda (Crustacea-Amphipoda), Mollusca, echinoderms and so on. The animal species reported in the present study (A. marina, S. armiger, P. elegans, N. diversicolor, M. baltica, Hydrobia) were recorded in some of the above studies, and details are given in Table 17.

Longbottom (1970) studied the distribution of A. marina on the north Kent coast with particular reference to the effects of particle size and sediment organic matter. She found that finer sediments contained more organic matter, and also a greater biomass of A. marina. She then stated that adult animals were found in the very finest sediments (median diameter $< 80 \mu\text{m}$), and attributed this to their inability to form burrows in these sediments. However, this latter statement should be treated with some caution, since Longbottom gives no data for particle size below $80 \mu\text{m}$.

Longbottom's results can be compared directly with mine from Ardmore - where A. marina were found. Her median particle sizes ranged from about $80 \mu\text{m}$ to about $200 \mu\text{m}$, her organic carbon values

TABLE 17. Comparison of the abundance (no./m²), biomass (g/m²), organic carbon and median particle diameter obtained from the present study with other references.

| Reference | | Locality geographical area | Nature of sediment (intertidal/subtidal) | Sediment type | No. of sites | Species that have been studied | Abundances (no./m ²) | Biomass | | Organic carbon | Median particle diameter (mm) |
|---------------------------|------|---|--|---|--------------|---|--|---|--|------------------------------|--|
| Author | Year | | | | | | | g/m ² | % of total | | |
| Cadée | 1976 | Dutch Wadden Sea | Tidal flats | Mud-dry sand | 2 | <u>A. marina</u> | 42.5-85 | | | 0.1-1.5% | |
| Holme | 1949 | Exemouth Estuary Britain pH: 7.5-8.0 Salinity: 0-34.5 ‰ | Intertidal | Mud-sand | 40 | <u>A. marina</u> <u>P. elegans</u> <u>N. diversicolor</u> <u>S. armiger</u> <u>M. baltica</u> | 1-60 1-2540 1-132 1-316 1-36 | 1.92-47.84 0.12-0.96 0.16 | 0.12-6.8% 0.26-1.3% 0.15% | | 95% of the total sample made up of grades ≤0.5 mm = ≤500 μm |
| Longbottom | 1970 | North Kent Coast/Britain | Intertidal | Fine mud-muddy sand | 10 | <u>A. marina</u> | 68 | 5-40 | | (0.04-1)% | 80-200 μm |
| Stephen | 1930 | Scottish coasts Britain | Intertidal | 1) River mud flat (sticky mud) 2) Grounds at the heads of lochs (sand) 3) Exposed shores on Western Island (sand) | 3 8 | <u>M. baltica</u> <u>N. baltica</u> <u>S. armiger</u> <u>N. baltica</u> <u>N. diversicolor</u> <u>S. armiger</u> <u>A. marina</u> | 156 24 4-20 8-20 (5 sites) 16 (1 site) 4-24 (4 sites) 68-76 (2 sites) | | | | |
| Whitlatch | 1981 | Barnstable Harbor Massachusetts | Intertidal | Mud-sand | 16 | <u>P. elegans</u> | (0.8-6.8)/core (203.2-1727.5)/m ² | | | (0.07-1.34)% | |
| Data in the present study | | A) Langbank | Intertidal | Sandy-mud | | <u>C. volutator</u> <u>N. diversicolor</u> <u>M. baltica</u> | 7127.8 (85%) 1044.8 (12.5%) 197.6 (2.5%) | 40.31 479.4 9.5 | 7.6% 90.6% 1.8% | 6.161 (mgC.g ⁻¹) | 1.7 ϕ = 250-355 μm |
| | | B) Ardmore | Intertidal | Sandy-mud | | <u>P. elegans</u> <u>S. armiger</u> <u>A. marina</u> <u>M. baltica</u> <u>H. ventrosa</u> <u>C. volutator</u> | 8983.9/m ² (92.3%) 298.8/m ² (3.1%) 33.66/m ² (0.35%) 55.60/m ² (0.57%) 296.5/m ² (3.04%) 64.20/m ² (0.66%) | 48.6 2.42 43 14.4 1.871 0.37 | 44% 2.2% 39% 13% 1.7% 0.34% | 5.312 (mgC.g ⁻¹) | 2.67 ϕ = 125-180 μm |

from 1% to 0.04%, and her biomass c. 40 to c. 5g/m² (fine to coarse sediment). The median diameter particle size at Ardmore is 2.76 ϕ \doteq 157 μ m, the organic content is 5.312 mg c/g dry sediment = 0.5312%, and the biomass of A. marina about 43 g/m² (Table 16, p.121; Table 13, p. 98; Figure 6, p. 68). In Longbottom's paper (1970) a median particle size of 148 μ m has an organic carbon content of c. 0.1 to 0.2% and a biomass of c. 20 to 30 g/m². In other words, the organic content and biomass of my sediment is higher than that of the equivalent particle size in Longbottom's study.

In a classic paper, Holme (1949) studied the abundance and biomass of a number of species at 40 sites near the mouth of the Exe Estuary. Holme's paper is very important, and I therefore discuss his results in some detail, relating them to my own investigations at Langbank and Ardmore.

Holme stated that the particle size of 95% of the total samples was made up of grades below 0.5 mm (= \angle 500 μ m). The median particle size at Langbank and Ardmore was 1.7 ϕ = 308 μ m and 2.67 ϕ = 157 μ m respectively, and the respective cumulative plots showed that 95% of the Langbank sediment was less than 2000 μ m and 95% of the Ardmore sediment was less than 250 μ m. Hence the Langbank sediment is coarser than, and Ardmore sediment finer than Holme's sediments. (Transformation of ϕ scale for particle size to μ m is shown in Appendix I, pp. 456, 457).

Holme (1949) did not measure organic carbon in his sediments and so I cannot compare my organic carbon results with the sediment studied by him.

The abundance of A. marina recorded by Holme (1949) ranged from 1 to 60 animals/m² and the biomass from 1.9 to 47.8 g/m² (0.12 to 6.8% of the total biomass). At Ardmore, I obtained abundance values of 25 to 39 animals/m² and biomass values of 31.9 to 49.8 g/m² (mean per cent of the total biomass = 39%).

Hence my abundance values for Arenicola marina fall within Holme's range, but the biomass and per cent biomass were higher in my study. The animals at Ardmore were therefore probably bigger than those at Holme's sites.

The abundance of P. elegans recorded by Holme (1949) ranged from 1 to 2540 animals/m², and the abundance of N. diversicolor ranged from 1 to 132 animals/m². However, he did not measure their biomass. At Ardmore, the abundance of P. elegans was 8983.9 animals/m². At Langbank, the abundance of N. diversicolor was 1044.8 animals/m².

In Holme's study, the abundance of Scoloplos armiger ranged from 1 to 316/m² and its biomass from 0.12 to 0.96 g/m² of the total. At Ardmore, the abundance of S. armiger was found to be 298.8 animals/m² and its biomass 2.42 g/m².

It is clear therefore that the abundance of N. diversicolor at Langbank and of P. elegans at Ardmore were higher than at Holme's sites, and the same is true for the biomass of S. armiger at Ardmore.

The general conclusion from this detailed comparison of Holme's (1949) sites with my Langbank and Ardmore data is that the sediments at my sites support a higher biomass and number of individuals. It is probable therefore that there is more food available to the

species at Ardmore and Langbank.

Having compared my results in detail with Holme's (1949) and Longbottom's (1970) work, I shall now discuss briefly the abundances and biomasses I obtained at Langbank and Ardmore.

At Langbank, C. volutator is by far the most abundant animal found at Langbank (85% abundance). However, the second most abundant animal, N. diversicolor (12.5% abundance), has the highest dry biomass (90.6% compared to 7.6% for C. volutator). This is due to the considerably larger size of N. diversicolor compared with C. volutator. M. baltica makes up only a small percentage of the animal population (2.5%) and has a correspondingly small dry biomass (1.8%).

At Ardmore, the animal population is more diverse than that found at Langbank. ^{I noted} Six species are present at Ardmore compared to three at Langbank.

P. elegans is the dominant species as far as abundance is concerned (92.3%). However, due to its small size it only forms 44% of the dry biomass. The largest animal, A. marina, only forms 0.35% of the population in abundance, but because of its large size forms a high percentage of the dry biomass (39%).

The four other species present have percentage abundance values lying between that found for P. elegans and A. marina.

C. volutator and M. baltica are the only species occurring at both sampling sites. However, C. volutator is the dominant animal at Langbank (85% abundance), while at Ardmore, it is relatively insignificant (0.66% abundance). A similar situation exists with M. baltica although the difference in abundance between sites is not so pronounced.

(II) Bacterial counts

There are many studies on the microbial ecology of coastal waters (Lynch and Poole, 1979; Nedwell and Brown, 1982; Rheinheimer, 1977; Stevenson and Colwell, 1973; ZoBell, 1946). However, there does not appear to be a great deal of microbial work conducted on the Clyde Estuary area (Anderson and Morris, 1974).

Estuarine water and sediment in the Clyde Estuary have been studied by Ross (1963) and Lloyd (1970). Sulphur bacteria have been investigated in relation to pollution in the Clyde area by Ellis (1925, 1926, 1929, 1932) and Ellis and Stoddart (1930). Bacteria, blue-green algae and microalgae have been studied in Clyde sediments by Anderson and Meadows (1969) and Meadows and Anderson (1966, 1968), and bacteria in the water column and sediments have been studied by Lloyd (1930, 1931). The only direct work on faecal pollutant bacteria in the Clyde sea area appears to be a short note by McCallum (1969), who estimated presumptive coliforms in water from various parts of the estuary. It is difficult to relate my work specifically to the above research work since none of the authors except Anderson and Meadows (1969) and Meadows and Anderson (1966, 1968) worked on intertidal sediments, and no one appears to have conducted comparative counts on marine, freshwater and coliform bacteria in this environment.

The counts of marine and freshwater isolates, and Escherichia coli-like organisms at the two sites studied are likely to be affected by a wide range of environmental parameters. Some of the more important ones are salinity (Langbank c. 26^o/oo and Ardmore c. 31.5^o/oo), organic input, particle size, and sewage load of the river.

Escherichia coli-like organisms as described by Cruickshank et al. (1975, pp. 125), grew on the teepol-lactose media (see Plate 5, p. 75). Results in Appendix I, Tables 10 and 11 show that teepol-lactose media supported comparatively few bacteria (Table 4, p. 82). This was true for both types of sediments. The number of bacterial colonies grown on the teepol agar medium was calculated as a per cent of that grown on the nutrient and bacto-marine agar media. These values were 11.9% and 2.4% for Langbank and 10.5% and 1.6% for Ardmore respectively. This is due to the presence of selective agents which are incorporated into the media.

Coliforms were present in greater numbers in Langbank than in Ardmore sediment (viable counts for Langbank were 72.71 times greater than for Ardmore). The probable reason for this is that Langbank is closer to the Clyde River and carries a lot of domestic and industrial sewage. Ardmore, on the other hand, is further down the estuary and is influenced more by the sea.

The distribution, survival, and significance of pathogenic bacteria and viruses in estuaries has been studied in a total of 72 water and sediment samples by Colwell and Kaper (1978). They related the decline or die-off of enteric microbial populations in the sea to several factors. These factors included dilution, sedimentation, predation, toxicity of trace metals in sea water, nutrient deficiencies, and salinity.

Another example is a study by Mitchell (1968) on the effect of water movement on lysis of non-marine microorganisms by marine bacteria. It has been demonstrated that the kill of non-marine microorganisms in the sea is directly related to the size of the

marine microbial population. In his discussion, he associated the kill of E. coli in the sea with two groups of microorganisms. The first was a group of bacteria mostly pseudomonads which produced extracellular enzymes active against the E. coli cell walls. The second was another group of halophilic filterable parasitic bacteria which were found to be stimulated by the addition of E. coli to sea water. Mitchell also found that non-marine fungi carried into the sea in run-off waters appeared to be similarly linked to the activities of the marine microflora. The rapid kill in areas of the sea where there was continuous flow of non-marine fungi into the sea due to the presence of an antagonistic microflora.

The viable counts determined on nutrient agar for Langbank sediment were greater than for Ardmore (viable counts for LBM were 64.28 times greater than for ARD). This could be explained by Langbank being closer to the Clyde River. The number of the freshwater forms will be reduced as one moves down the estuary towards the sea - that is, towards Ardmore. Particle size may also be important - Langbank is a slightly more muddy sediment than Ardmore.

The viable counts determined on bacto-marine agar for Langbank sediment were greater than for Ardmore sediment (the figure for Langbank was 49.28 times greater than that for Ardmore). This result is at first surprising since Ardmore is influenced by the sea to a greater extent than the Langbank site. One explanation for these findings is that some of the coliforms (introduced from industrial and domestic sewages) could have grown on the marine agar. The enhanced organic input from pollution and estuarine sedimentation of freshwater organic material will also be important, and will have stimulated growth.

However, from Table 4 (Results Section, p. 82), one can see that the ratio of marine bacteria to freshwater bacteria for Ardmore was greater than that for Langbank (6.767 and 4.927 respectively). These values reflect the nature of both Ardmore and Langbank sediments and underline the importance of salinity (salinity at the two sites was 31.5^o/oo and 26^o/oo respectively).

(III) Chemical measurements

1. Salinity

Salinity fluctuations in the intertidal area have been recorded by many authors (Barnes, 1974; Dyer, 1979; Meadows and Campbell, 1972, 1978). Different ranges were given by each of the above authors: 0-33^o/oo (Meadows and Campbell, 1972); less than 1^o/oo to above 35^o/oo (Meadows and Campbell, 1978); < 0.5^o/oo to \approx 35^o/oo to 37^o/oo (Barnes, 1974); and between river water and seawater salinity (35^o/oo) (Dyer, 1979).

My salinity results show that in general, Ardmore water samples had higher salinity values than Langbank water samples. This is because Ardmore is nearer to the sea than Langbank. The results also show that the overlying and channel water samples had higher salinity values than interstitial water for both Langbank and Ardmore samples. A possible explanation is that interstitial water in these sediments is partly derived from subterranean land run-off.

The lowest and highest salinity values recorded in this survey for the three types of water samples at the two sites were between 24.1^o/oo and 31.1^o/oo (Table 6, p. 85). These two values are within the ranges quoted by other authors (see above).

2. pH

There have been a number of studies on pH in which Eh has also been measured (see Introduction section, p. 26 for references and details of the relationship between the pH and Eh).

For example, Moshiri and Crumpton (1978) studied some aspects of redox trends in the bottom muds of a mesotrophic estuary. Their study included redox profiles, pH, bacterial numbers and organic content of the sediments. These data were accompanied by field determinations of dissolved O_2 , pH, temperature and salinity. Their results pointed to the importance of bacterial activity and dissolved - oxygen in the bottom - water on the redox status of the sediment.

In my studies, Ardmore water samples had higher pH values than those at Langbank. Overlying and channel water samples had higher pH values than the interstitial samples at both sites. A slight increase in sediment pH with depth was found for both Langbank and Ardmore sediments.

The low pH of the interstitial water relative to the overlying water may be due to the following factor. H_2S is produced by microorganisms during the anaerobic oxidation of organic matter (Friedman and Sanders, 1978; Stanier et al., 1976). This H_2S may be in some way associated with the lower pH's of the interstitial water.

Recorded values of the pH of sediments in the literature appear to be rather varied. For example, ZoBell (1946) records a range of 6.4 to 9.5 and Bågander and Niemistö (1978) a range of 6.9 to 8.3. My data at Langbank and Ardmore fall within these ranges, being between 7.7 and 9.2. The first of these authors

(ZoBell, 1946) records a slight increase in pH with depth into the sediment, while the second authors (Bågander and Niemistö, 1978) state that their surface sediment had a pH of 7.5 to 8.3 and subsurface sediment a pH of 6.9 to 7.5. My own studies show a slight increase with depth into the sediment and therefore appear to agree with ZoBell's data.

3. Eh

There are a number of studies on Eh profiles in near shore and estuarine environments (see Introduction, p. 26 for references). Broadly speaking they indicate that Eh is high at and within a few centimetres of the sediment surface. The Eh then falls over the depth range 2-10 cm, often becoming anaerobic with H₂S production in organically rich sediments. There appear to be no specific references to the Eh of overlying water in estuarine conditions.

ZoBell (1946) was probably the first person to show that Eh decreases with increasing depth into the sediment. Since then there have been many similar studies. For example, Bågander and Niemistö (1978) found rapid vertical changes of redox potential over few millimetres in sediments. At the oxidised layer at the sediment-water interface, redox values between +350 to +550 mV were obtained. A few centimetres deeper there were abrupt changes in the redox potential. The Eh values recorded in this layer were between 0 to +200 mV. This is sometimes called the redox discontinuity layer (Fenchel and Riedl, 1970). In the Gotland Deep (F81) and Farö Deep (F80), Bågander and Niemistö (1978) found that the bottom water usually had a low oxygen content, and

was periodically totally depleted of oxygen. The sediment surface was reduced and the redoxcline was situated just at or above the sediment-water interface. The Eh values lay between -100 and -200 mV. Hydrogen sulphide produced by sulphate-reducing bacteria probably control the redox potential here (Bågander and Niemistö, 1978).

In this context it is interesting to note that positive Eh values are generally characteristic of bottom deposits which are oxygenated, or which consist of coarse sediments or are poor in organic matter (ZoBell, 1946). Negative Eh values are characteristic of bottom deposits rich in organic matter and which consist largely of fine sediment.

Generally, oxygen is high at the sediment surface and decreases with depth. A good example of this phenomenon is the presence of a black layer below the sediment surface caused by anaerobic conditions (Anderson and Meadows, 1978; Moshiri and Crumpton, 1978). This layer was also described by Fenchel and Riedl (1970). I noticed the presence of the black layer in all samples collected from Langbank and Ardmore sediments during the present study.

At Langbank and Ardmore, high Eh values were recorded at the sediment surface (+410 and 290 mV respectively), Eh decreased to its lowest value at 5 cm depth (180 mV for LBM and 226 mV for ARD), but then increased again at deeper depths (Fig. 10, p. 92). Fenchel and Riedl (1970) recorded a value of +400 mV at the sediment surface, and around +200 mV at the deeper layers. Below this zone, there was a 'grey zone' or RPD layer. Oxygen, as well as reduced compounds such as hydrogen sulphide, were present

in small amounts. In this layer, Eh decreased quickly from positive to negative values. In the third layer, 'the black layer' or the 'sulphide zone', Fenchel and Riedl (1970) found that oxygen was totally absent while H_2S occurred in large amounts. They recorded Eh values in the interstitial water in this layer of between -100 and -250 mV. They also noticed that the sediment was black due to the presence of iron sulphide.

The increase of the Eh values at the deeper depths in both sediments may be caused by several factors. One of these might be subterranean flow of land drainage water. Another might be coarser sediments deeper in the sediment. For example, at depths greater than 20 cm it was noted that the sediment was coarse and contained a lot of gravel compared to the near surface sediment. This made insertion of the core sampler into the sediment difficult. A coarse sediment will have a higher porosity and water content, and therefore is likely to have a higher Eh because water will move through it more easily. ZoBell (1946) noted this phenomenon (see p. 133).

Bioturbation (Cadée, 1976; Gust and Harrison, 1981; Moore, 1958; Ott et al., 1976; Schäfer, 1972) is also likely to effect Eh values in sediments because burrows will be ventilated by animals living in them. Bioturbation is a well known phenomenon (Ott et al., 1976; Rhoads, 1974; Winston and Anderson, 1971) but there appears to be no work on studying how it affects Eh, except for recent measurements in deep sea sediments (Meadows and Tait, 1985). I did not attempt to relate bioturbation to my Eh values, and can therefore make no comments on its possible importance at Langbank and Ardmore.

The overlying and channel water samples at Langbank and Ardmore had higher Eh values than the interstitial water samples. The values for the overlying water samples at both sites were 400, 345 mV for Langbank, and 356.7, 360 mV for Ardmore. The Eh value of the interstitial water at Langbank and Ardmore was 260 and 256.7 mV respectively. The higher Eh values of the overlying and channel water samples at both sites can be explained as follows. The overlying and channel water at both sites is replenished regularly by river water and by sea water brought in by the tide. These two types of water will contain much higher oxygen levels than interstitial waters.

4. Organic carbon

There are many methods and literature references to the analysis and estimations of carbon content of sediments (see Introduction, p.27 for references). Organic carbon determinations of intertidal and subtidal marine sediments have been conducted in a number of studies (Aller and Yingst, 1980; Anderson and Meadows, 1978; Biernbaum, 1979; Cadée, 1976; Deans, Meadows and Anderson, 1982; Longbottom, 1970; Rhoads and Young, 1971; Whitlatch, 1981; Young and Rhoads, 1971). Measurements of organic carbon in these studies were conducted for a number of reasons. Some authors were interested in the relationship between particle size and organic carbon in order to relate this to the abundance and biomass of different species. Others were interested in organic carbon in relation to sediment selection by burrowing organisms.

Cadée (1976) studied sediment reworking by Arenicola marina on tidal flats in the Dutch Wadden Sea. He found that A. marina was the largest and quantitatively most abundant deposit feeder

in his study area. A. marina preferred to ingest sediment particles smaller than 300 to 400 μm . Concentration of coarse particles was found to be high at the feeding depth. Cadée (1976) found high values of organic carbon and organic matter at the feeding depth due to concentration of indigestible peat detritus at that level.

Anderson and Meadows (1978) studied the microenvironments in marine sediments. They recorded organic values for the three sediment layers which they recognised. These values for the brown, black and grey layers were 2.30, 5.17 and 1.31 (mg/g dry weight) respectively. Since Anderson and Meadows (1978) gave no values for the particle size analysis of their sediment, I cannot compare their results with the recorded organic content in my study.

Deans, Meadows and Anderson (1982) studied the physical, chemical and microbiological properties of the intertidal muddy sediment at Langbank. They recorded an organic carbon value of 10.66 mg.c/g dry weight and a mean particle size of 255.3 μm . The recorded organic carbon and median particle size values at Langbank in the present study were 6.161 mg.c/g dry weight and 308 μm respectively (see Table 13, p. 98 and Table 16, p. 121). The differences in the organic carbon content from the above study by the three authors in 1982 and that in the present study can be explained as follows. The high organic carbon content of 10.66 mg.c/g dry weight recorded in the study in 1982 was probably due to the fine particle size (255.3 μm).

The results of my work showed that both natural sediments (LBM and ARD) had higher organic carbon contents than Rockware sediment. Langbank sediment had a slightly higher organic carbon content than Ardmore. The higher value for Langbank sediment may be caused by the input of sewage to the estuary at a point close to the Langbank sampling area, and by precipitation of river-borne dissolved organic material.

For all sediment types, ashing appears to be more effective in removing the organic carbon than acid-cleaning. The ashing treatment may be more effective than the acid-cleaning treatment in determining organic carbon content of sediment for the following reasons. Carbon exists in many forms in sediments including coal, graphite, carbonate materials, soluble salts such as HCO_3 and remains of living organisms (Gross, 1971). Acid-cleaning relies on the breaking of specific chemical bonds for the oxidation of carbonaceous materials, while few materials are resistant to oxidation by ashing at high temperature (600°C).

(IV) Physical measurements

1. Shear strength

The increase of shear strength with depth is a well known engineering concept (Capper and Cassie, 1976; Lambe and Whitman, 1979), and is due to an increase in overburden pressure with depth. That is, deeper sediment particles are compacted together by sediment lying above them so that intergranular friction between the particles is increased so increasing the shear strength. Residual shear strength values are generally lower than peak shear strength values since residual measurements record the shear strength of sediment after failure or shearing.

Shear strength has been recorded in a number of geotechnical studies of near shore and estuarine sediments (Bokuniewicz, Gordon and Rhoads, 1975; Deans, Meadows and Anderson, 1982; Hagerty, 1974; Holmes and Goodell, 1964; Keller, 1974; Lee, 1982; McMaster, 1967; Moore, 1964; Richardson and Park, 1976; Rowe, 1974), but all of these except one were investigations of subtidal sediments. Deans, Meadows and Anderson (1982) investigated the penetrability of intertidal sediment at Langbank using a falling steel ball, but this method is not strictly comparable with the vane method I used at the same site (Meadows - personal communication), and only measures penetrability of the top 1-2 cm. My vane measurements extended to one metre depth.

The shear strength results showed that, in general, residual shear strength values were lower than peak shear strength values for both Langbank and Ardmore sediments (Fig. 11, p. 102 and Fig. 12, p. 104). At Langbank, shear strength increased progressively with depth (Fig. 11, p. 102). At Ardmore, shear strength increased to a depth of 45 cm, then decreased (Fig. 12, p. 104). The highest peak shear strength value recorded during the survey was found at Ardmore (0.350 kg/cm^2 at a depth of 45 cm).

The decrease of shear strength at Ardmore below a depth of 45 cm may be caused by several factors. A change in particle size and water content may occur at this depth. Both of these properties affect shear strength (Jumikis, 1962, Chapter 19, p. 475), since they affect cohesion and friction between sediment particles.

In general, high water content sediments with a large mean particle size have a lower shear strength than sediments with a low water content and a small mean particle size (Trask and Rolston, 1950). But Ardmore sediment is a very fine sediment (i.e. clay)

at depths greater than 10 cm, and would therefore be expected to have a higher shear strength (Trask and Rolston, 1950). However, clays have unusual properties that might produce a reduction in shear strength: slip may occur more easily between particles of clay than between ordinary sediment particles because clay is made up of plate-like particles. It is, therefore, interesting to note that Moore (1964) found inverse relationship between shear strength and the percentage of clay sized particles in sediments at six out of seven deep sea sites near Guadalupe and in the San Diego Trough in the Pacific.

The unexpected decrease in shear strength below a depth of 45 cm may also be related to bioturbation. Arenicola marina burrows are present at Ardmore and can extend to depths greater than half a metre. Schäfer (1972) reported A. marina burrows extending to depths of around 40 cm. The decrease in shear strength may be due to the vane of the shear strength apparatus encountering an Arenicola marina burrow. A. marina is not found at Langbank, and a more uniform increase in shear strength with depth is found at that site.

2. Particle size analysis

Many methods have been used to analyse and describe the particle size distribution of sediments (Allen, 1975; Bagnold and Barndorff-Nielsen, 1980; Barrett, 1980; Burger, 1976; Folk and Ward, 1957; Folk, 1966, 1980; Galenhouse, 1971; Inman, 1952; Krumbein and Pettijohn, 1938; Soggi and Tanner, 1980; Tanner, 1964; Winkelmoen, 1982). These methods vary in their accuracy, their pictorial presentation and the way the data is transformed.

In general, the methods used are either graphical, for example data derived from cumulative frequency curves and histograms as

described by Inman (1952) and Allen (1975) respectively, or mathematical such as the method of moments described by Folk (1980). I used Inman's (1952) graphical method.

The results of the particle size distribution of Langbank, Ardmore and Rockware sediments using Inman's (1952) method can be summarised as follows (see Table 16, p. 121).

The largest mean particle size was found with Langbank sediment ($1.7\phi = 308 \mu\text{m}$), followed by Rockware sediment ($2.0\phi = 250 \mu\text{m}$) and the Ardmore sediment ($2.67\phi = 157 \mu\text{m}$). The best degree of sorting, that is, lowest standard deviation, was found with Rockware sediment ($0.325\phi = 798.3 \mu\text{m}$), followed by Ardmore sediment ($0.48\phi = 717.0 \mu\text{m}$) and then Langbank sediment ($0.65\phi = 637.3 \mu\text{m}$). Positive skewness was found in all three sediment samples. The greatest skewness was found with Ardmore sediment (0.1458), followed by the Langbank sediment (0.07692), then Rockware sediment (0.06154). The highest value of kurtosis was found with Langbank sediment (1.115), followed by Rockware sediment (0.9692) and then Ardmore sediment (0.6146). Skewness and kurtosis are non-dimensional parameters, and hence have no units.

Folk (1980) provides qualitative descriptions of sediments in terms of mean particle size, sorting, skewness and kurtosis. The three sediments have been classified according to these descriptions in Table 18.

Mean and median are measures of central tendency of the particle size distribution. Since ϕ is equal to $-\log_2 \text{mm}$ (Inman, 1952), high ϕ values represent small particle sizes. The largest mean and median particle sizes were found with Langbank (mean = 1.7ϕ , median = 1.75ϕ), followed by Rockware sediment (mean =

2.0 ϕ , median = 2.02 ϕ). Ardmore sediment had the smallest mean and median particle sizes (mean = 2.67 ϕ , median = 2.74 ϕ). According to Folk (1980) (Table 18), both Ardmore and Rockware sediments are classified as a fine sand whereas Langbank sediment is a medium sand.

Standard deviation or sorting is a measure of the number of particle size classes present in a sample. A high standard deviation means a poorly sorted sediment (i.e. there are a lot of different particle sizes present). A low standard deviation, means a well sorted sediment (i.e. there are only a few different particle sizes present).

The highest standard deviation was found with Langbank sediment (0.65 ϕ), followed by Ardmore sediment (0.48 ϕ) and then Rockware sediment (0.325 ϕ). Therefore, Rockware and Ardmore sediments are very well sorted and well sorted sediments respectively, while Langbank is moderately well sorted.

Normally, if a sample is poorly sorted, it indicates that erosion by water currents is less significant than in a sample which is well sorted. Since Ardmore sediment is well sorted and Langbank sediment is moderately well sorted, this would indicate that erosion is more significant at Ardmore than at Langbank.

Skewness is the extent of departure of the mean from the median (Inman, 1952). In a normal distribution, the mean is equal to the median, therefore, no skewness is present and the distribution is symmetrical. If the distribution deviates from normality the mean and median diverge. A distribution can be positively or negatively skewed. A positively skewed distribution is one in which greater amounts of fine material occur than would

be expected in a normal distribution (Duane, 1964). A negatively skewed distribution has relatively more coarse material. Since all three of my samples were positively skewed, they contain relatively more fine material than would be found in a normal distribution.

The highest skewness value was found with Ardmore sediment (+0.1458), followed by the Langbank sediment (+0.07692) and then Rockware sediment (+0.06154). Using Folk's (1980) description, Langbank and Rockware sediments are both near symmetrical skewed, while Ardmore sediment is finely skewed.

Duane (1964) studied the significance of skewness in recent marine sediments in western Pamlico Sound, North Carolina. He determined the skewness of many samples from both exposed and sheltered marine environments. Duane regards skewness as being environmentally sensitive. He found that sediments in the littoral zone, beaches and tidal inlets are all negatively skewed, and stated that negative skewness is due to winnowing or erosion processes. Negatively skewed sediments were found in areas where erosion was dominant over deposition. Positively skewed sediments were indicative of areas where sediment deposition was dominant over sediment erosion. Duane (1964) suggested that areas characterised by no particular dominance of either positive or negative skewness were regions where winnowing might be effective one day but not the next. Both Langbank and Ardmore sediments were positively skewed - Ardmore more than Langbank. Hence sediment deposition is dominant over erosion at both sites, but more so at Ardmore than Langbank.

In this context, it is interesting that Ardmore is well sorted (low s.d.) while Langbank is moderately well sorted (higher s.d.), and so erosion appears to be greater at Ardmore than at Langbank. This is probably caused by greater wave and tidal action at Ardmore than at Langbank because Ardmore is closer to the mouth of the estuary.

Kurtosis as defined by Inman (1952) is a measure of the flatness or peakedness of a curve. Langbank and Ardmore sediments are leptokurtic and very platykurtic respectively, indicating that their particle size distributions are respectively slightly peaked and flatter than a normal curve (Folk, 1980). Rockware sediment is mesokurtic which represents the normal distribution. It is difficult to suggest sensible ecological reasons for the difference between the Langbank and Ardmore kurtoses.

TABLE 18. Qualitative descriptions (Folk, 1980) used to describe Langbank (LBM), Ardmore (ARD) and Rockware (RWS) sediments in terms of mean particle size, sorting (s.d.), skewness and kurtosis.

| Factors | Sediment type | | | Folk (1980) PP. |
|---------------------|--|---------------------------------|-------------------------------------|-----------------|
| | LBM | ARD | RWS | |
| Mean particles | Medium sand 1.0-2.0 ϕ | Fine sand 2.0-3.0 ϕ | Fine sand 2.0-3.0 ϕ | 23 |
| Standard deviations | Moderately well sorted 0.50-0.71 ϕ | Well sorted 0.35-0.50 ϕ | Very well sorted < 0.35 ϕ | 42 |
| Skewness | Near symmetrical (+0.10)-(-0.10) | Fine skewed (+0.30)-(+0.10) | Near symmetrical (+0.10)-(-0.10) | 44 |
| Kurtosis | Leptokurtic 1.11-1.50 | Very platykurtic < 0.67 | Mesokurtic 0.9-1.11 | 45 |

SECTION I

Summary

1. Animal abundance and animal biomass

The animal abundance and animal biomass were measured from three small and three large cores collected from Langbank and Ardmore sediments.

Data were recorded for the number of animals and wet and dry biomass in the top (0-10 cm) and lower (10-20 cm) parts of each core. Most species were only found in the top 10 cm of sediment. Exceptions were N. diversicolor and M. baltica at Langbank and A. marina at Ardmore which was found at depths greater than 20 cm.

At Langbank, C. volutator was the most abundant species and made up about 85% of the total number of animals followed by N. diversicolor (12.5%) and then M. baltica (2.5%). In terms of dry biomass, N. diversicolor was found to be the most dominant species followed by C. volutator and then M. baltica.

At Ardmore, P. elegans was the dominant species and formed about 92% of the total number. The other species were rare in comparison. The dry biomass showed that P. elegans and A. marina were the dominant species followed by M. baltica, S. armiger, H. ventrosa and C. volutator.

2. Bacterial counts

Viable counts of bacteria were determined using three types of nutrient media. Samples were collected by removing the top one centimetre of surface sediment from both Langbank and Ardmore.

Three media were used: nutrient agar (a freshwater medium), bacto-marine agar 2216 and teepol-lactose agar. Two series of

experiments were carried out, the first was preliminary and the second definitive.

Colony forming unit (C.F.U.)/g of dry sediment were calculated from those plates having between 30 and 300 colonies.

The number of bacteria recovered from Langbank sediment on all the three media was higher than from Ardmore sediment.

The results from the definitive experiment showed that at both sites, the highest number of bacterial colonies grew on the bacto-marine agar 2216 followed by the nutrient agar and then the teepol-lactose agar.

3. Chemical factors

The chemical factors measured were salinity, pH, Eh and organic carbon.

- a. Higher salinity values for the three types of water were recorded at Ardmore than at Langbank. At both sites, the interstitial water sample had a lower salinity value than the other two water samples, overlying and channel.
- b. pH values for the three types of water showed that Ardmore water samples had slightly higher pH values than the equivalent water samples at Langbank. The interstitial water at both sites had a slightly lower pH than the overlying and channel water samples.
- c. The Eh values of the overlying and channel water samples at both sites were higher than the Eh values of the interstitial water.

- d. pH and Eh for both Langbank and Ardmore sediments were measured to a depth of 40 cm.

A slight increase in pH with depth was found for both sediments. The pH in this study lay between pH 7.7 and pH 9.5.

Eh values for both sediments were relatively high at the surface and then decreased at the depth of 5 cm. At 10 cm depth, Eh increased again and remained relatively constant to a depth of 40 cm. At Langbank, Eh at the surface was found to be higher than at Ardmore.

- e. Organic carbon. The organic carbon of Langbank, Ardmore and Rockware sediments were measured using the wet oxidation method. The organic carbon content of Langbank and Ardmore sediments were higher than Rockware sediment.

Two methods were tested to find their effects on removing organic carbon from sediments. The two cleaning methods were ashing and acid-cleaning. Ashing and acid-cleaning methods dramatically reduced the organic carbon levels in the three sediment types. Although the percentage losses of organic carbon after ashing and acid-cleaning were much higher in Langbank and Ardmore sediments than in Rockware sediments, all the absolute values in mg.c g^{-1} sediment were still significantly higher in Langbank and Ardmore sediments than in Rockware sediment. Ashing appears to remove more organic carbon from both natural sediments than acid-cleaning. This effect was not significant in Rockware sediment.

4. Physical factors

The physical factors measured in this study were shear strength and particle size.

- a. Shear strength. The shear strength of Langbank and Ardmore sediments were measured to a depth of one meter using a Pilcon Hand Vane Tester. The residual shear strength values (kg/cm^2) were lower than the peak values for both types of sediment. Shear strength values of Langbank sediment increased progressively with depth. With Ardmore sediment, shear strength increased to a depth of 45 cm, then decreased.
- b. Particle size analysis. Particle size analysis was carried out on the three sediments. Ordinary and cumulative plots of the particle size were made. Parametric analysis of Langbank, Ardmore and Rockware sediments were carried out using Inman's (1952) method.

Ardmore sediment had the smallest median and mean diameters followed by Rockware and then Langbank. The median diameters were slightly less than the mean diameters. Langbank sediment had the greatest standard deviation followed by that of Ardmore and then that of Rockware. Ardmore sediment had the largest skewness followed by Langbank and then by Rockware, while Langbank sediment had the largest kurtosis followed by Rockware and then Ardmore.

According to the degree of sorting, Langbank, Ardmore and Rockware sediments were classified as moderately well sorted, well sorted and very well sorted respectively.

In terms of skewness, Langbank and Rockware were classified as near-symmetrical and Ardmore as fine-skewed. The kurtosis descriptions of Langbank, Ardmore and Rockware sediments were leptokurtic, very platykurtic and mesokurtic respectively.

A parametric analysis of four normally distributed theoretical size distributions has been conducted using Inman's (1952) method. The values of the parameters, and the graph plots, of the four arbitrarily chosen straight lines, each of which represents a different normally distributed particle distribution, are presented in Appendix I (pp. 451).

SECTION II

CHEMICAL AND PHYSICAL EFFECTS ON SEDIMENTATION

SECTION II

IntroductionGeneral

In this section, I describe the details of a number of estuarine processes relevant to my work. The reason that these details are presented in this Introduction rather than in the introductions to Section I (p. 23) and Section III (p. 278) is because I consider them to be more relevant to the physical and chemical effects on sedimentation described in this section and to the effects of benthic animal secretions described in the following section (Section III, p.278-).

The estuarine processes that are described in the following introduction are as follows.

- (I) Sedimentation in estuaries.
- (II) Sediment transport in estuaries.
- (III) Theoretical aspects of sediment and related phenomena.
- (IV) Recent work on sediments and sedimentation.
- (V) Aspects of sedimentation studied in Section II and Section III of the thesis.
- (VI) Aims of the work presented in the present section.

(I) Sedimentation in estuaries

Estuarine processes are not simple. The river discharge is mixed with sea water by the action of tidal motion, by wind on the surface and by the river discharge moving towards the sea. Each of these mixing processes acts with a different time scale of variability (Dyer, 1979; Perkins, 1974; Reineck and Singh, 1980).

The salinity difference between the river and the sea water is about 35⁰/oo creating a difference in density of about 2%. Even though this is small, it is sufficient to cause horizontal pressure gradients within the water which affect the way it flows. Mixing of river and sea water causes precipitation and flocculation of dissolved organic matter and fine colloidal organic particles and clay (Sholkovitz, 1976; Straaten and Kuenen, 1958). Fine grained material will move in suspension and will follow the residual water flow, although sediment deposition may occur during slack water. The coarser grained material will travel along the bed and will be affected most by high velocities and will move in the direction of the maximum current (Dyer, 1979; Perkins, 1974).

Further details of fine and coarse grained material will now be considered under separate headings.

1. Fine particles

Particles smaller than about 2 μm in diameter are mainly composed of the clay minerals illite, kaolinite and montmorillonite. These particles are liable to collide and to flocculate into larger aggregates in sea water (Dyer, 1979; Sholkovitz, 1976; Straaten and Kuenen, 1958). The concentration of the particles and electrolytes in water affects this process. The explanation of those physico-chemico processes is as follows.

Elementary particles of colloidal or semi-colloidal dimensions may have an electric charge which influences their behaviour in suspension. Of these particles, clay minerals are of primary importance (Postma, 1967). It has been found that these minerals usually have negative charges. This charge may be explained by: (1) preferential adsorption of anions, especially hydroxyl ions, (2) cationic substitutions within the crystal lattice, and (3)

residual valences (broken bonds) at particle edges (Chambers' Encyclopaedia, 1950, p. 631). The negative charge is balanced by a double layer of hydrated cations which tend to move away from the surface of the clay mineral, although electrostatic attraction prevents a complete escape. There is a correlation between the stability of the suspension and the electrolytic potential (i.e. the thickness of the double layer) (Postma, 1967). The thickness of the double layer depends on the valence of the sorbed ions, the total ion concentration in the surrounding water, temperature and pH. If the electrolytic potential decreases below a critical value, flocculation occurs. The probability of flocculation occurring is related to particle concentration and to electrolyte concentration. With adequate particle concentrations, flocculation of illite and kaolinite is complete above a salinity of about 4⁰/oo (Barnes, 1974; Dyer, 1979). However, for montmorillonite, the floc size varies over the entire salinity range (Whitehouse et al., 1960). The size of the flocs may also be reduced by turbulence (Dyer, 1979) (Flocculation is discussed in more detail on p. 262.)

A high concentration of particles in suspension can decrease turbulence. Here, the floccules will not fall as separate units but as layers (Dyer, 1979). After the suspended particles have settled, the weight of the new overlying sediment forces out the pore water and the floc structure slowly collapses (Partheniades, 1965). The rearrangement of the particles gives the bed increased shear strength and resistance to re-erosion.

2. Coarse particles

Coarse particles are composed of sand and gravel-size material that does not flocculate. This bed load material will move downstream to the tip of the saline intrusion. There, as the maximum

currents are equal on ebb and flood at the bottom, the material will become deposited. Further seaward, the maximum landward flood current will move the bed material into a position where the velocities do not exceed the threshold for the grain size (Dyer, 1979). Consequently there should be a decrease of grain size inland. However, this is a rather idealised picture as the tidal currents are not simply distributed. The height to which a sediment particle is carried into suspension is dependant upon the turbulence of the water (Hjulström, 1939) (as is described by Perkins, 1974). The settlement velocity is influenced by the size, shape and specific gravity of the medium through which they settle (Green, 1968; Perkins, 1974).

(II) Sediment transport in estuaries

1. Justification

My sedimentation experiments are clearly of direct relevance to sediment transport in estuaries, since the more slowly particles settle the further they will be transported in any current.

2. Describing the process

The dynamics of sediment transport in moving water are not simple (Dyer, 1979; Frostick and McCave, 1979; Grant, 1981; Green, 1968; Lambiase, 1980; Larsen et al., 1981; Perkins, 1974; Postma, 1967; Reineck and Singh, 1980). Suspended matter is carried back and forth, and is deposited and eroded many times before it finally settles (Perkins, 1974). The transport of sediment by water movement in estuarine areas is caused by river and tidal currents, wave action, and longshore drift (Meadows and Campbell, 1978; Perkins, 1974). Relationships between erosion, transportation and deposition velocities and the grain size of sediments have been developed by Hjulström (1935) and re-examined by Sundborg (1956).

The critical erosion velocity is the minimum current velocity at which sediment of a particular size begins to move; the movement stops at a flow velocity called the lowest transportation velocity or the deposition velocity (Postma, 1967). Erosion, transportation and deposition for various grain sizes are illustrated in Figure 21 (Postma, 1967). In Figure 22, I have redrawn the original graphs presented by Hjulström (1935) and Sundborg (1956), on which Figure 21 is based. The reason for doing this is that the graphs in the original references (Figure 22) are significantly different from the data as presented in many modern accounts (e.g. Figure 1; Postma, 1967).

Figure 21

Erosion, transportation, and deposition velocities for different grain sizes. The diagram indicates possible values for various stages of consolidation. (From Postma, 1967, p. 158.)

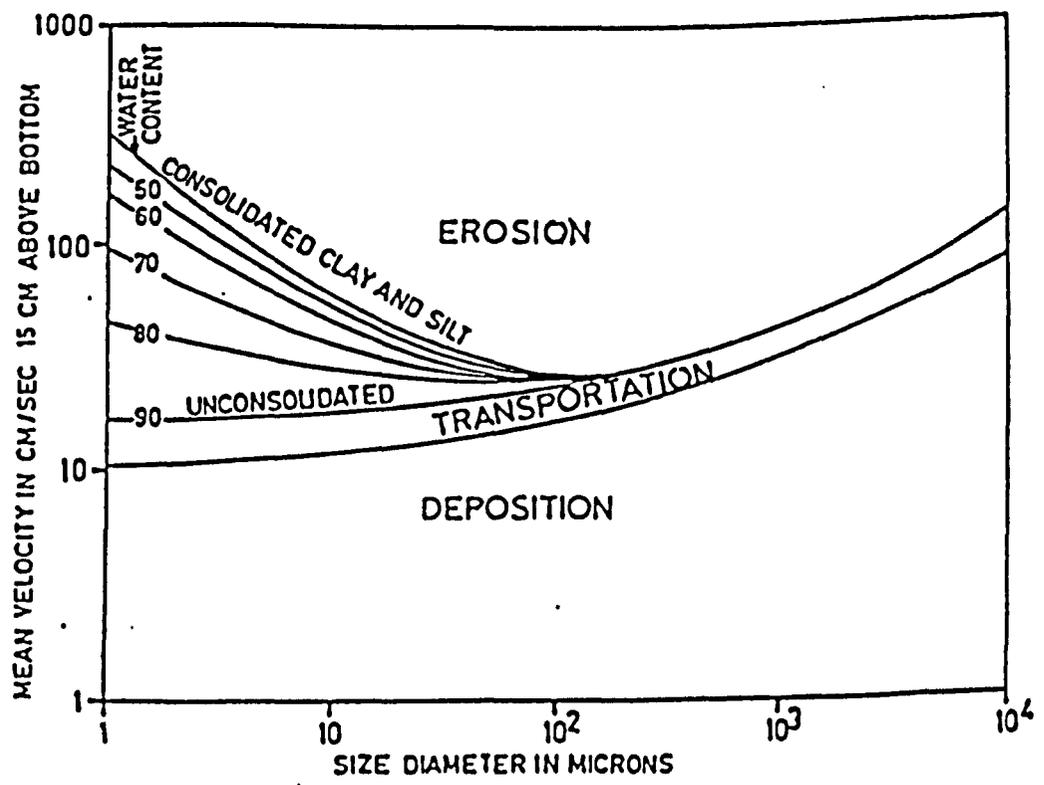
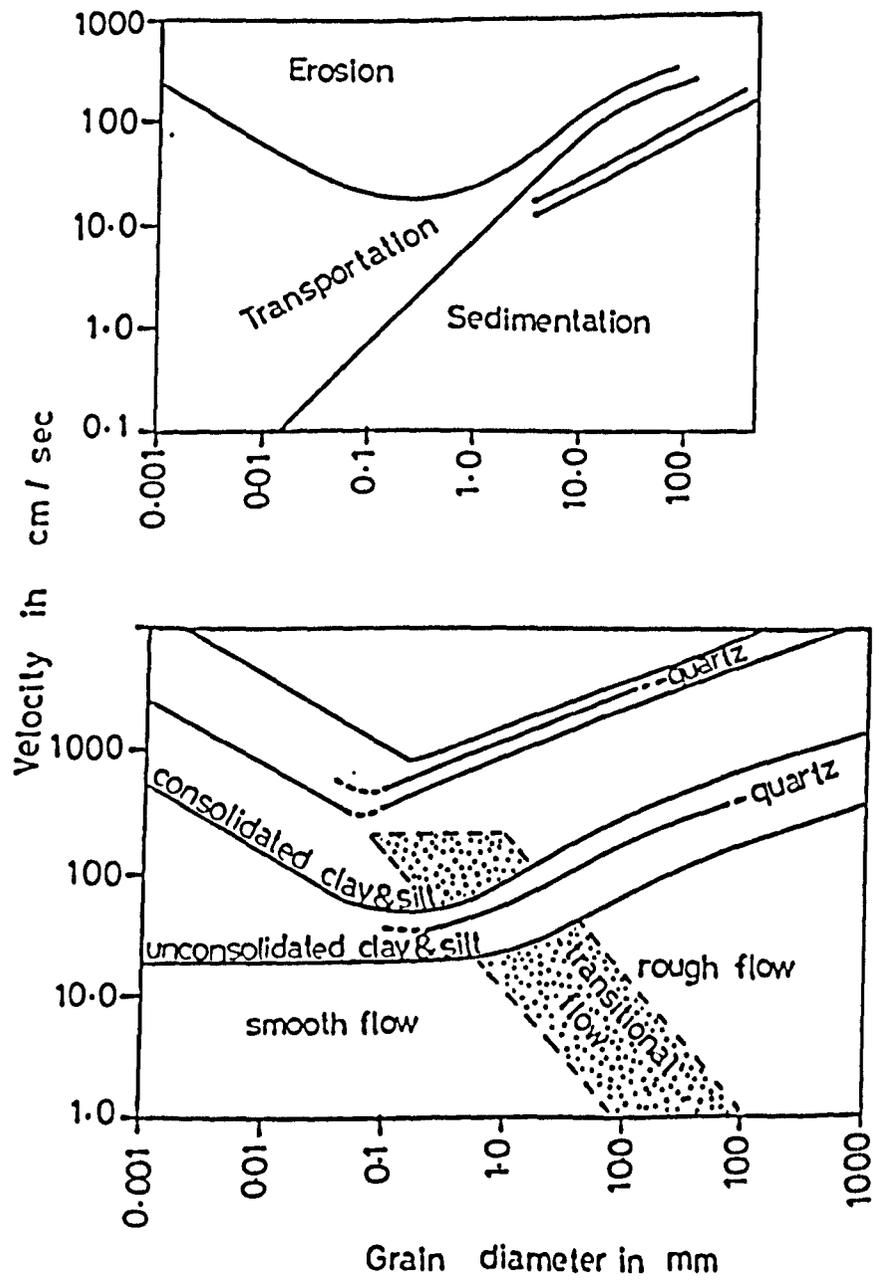


Figure 22

Erosion, transportation, and deposition velocities for different grain sizes. The top picture was given by Hjulström (1935) and the re-examined picture by Sundborg (1956). Note Log_{10} scale on both axes.



Larsen et al. (1981) investigated the threshold of grain motion produced by ocean waves and currents under field conditions. They concluded that Shields entrainment function for unidirectional flow can be used to predict the threshold of grain motion for oscillatory flow conditions on the continental shelf.

Shields entrainment function (Shields, 1936) written in terms of fluid velocity is:

$$\Psi_m = \frac{\rho U_m^2}{(\rho_s - \rho)gD}$$

Where ρ_s and ρ are the sediment and fluid densities, respectively, g is the acceleration due to gravity, and D is the mean grain diameter. U_m is the fluid velocity at a designated distance (usually 1 m) above the seabed. The Shields criterion expresses a critical value of the ratio of the entraining force to the stabilising force acting on a sediment grain. The entraining force is related to the shear stress exerted on the bed by the moving fluid, the stabilising force is related to the submerged weight of a sediment grain. When the ratio of the two forces exceeds a critical value, sediment movement is initiated. This criterion is a dimensionless relationship which is quite general in that it applies for any fluid flow and sediment characteristics so long as the sediment is cohesionless.

Sedimentary materials may be transported in a number of ways depending upon the water velocity (Hjulström, 1935; Perkins, 1974; Reineck and Singh, 1980; Sundborg, 1956). With increasing velocity, particles may move by sliding, rolling, saltation and suspension (Perkins, 1974, p. 133). Sliding is rarely seen, whereas rolling is the normal mode of transportation in rivers; in these instances,

the particle's velocity is much less than that of the water. With a further increase in velocity some of the particles undergo a hopping motion called saltation. In saltation, some of the particles are lifted into suspension but only in a part of the path of motion does the particle have the velocity of water. When transportation by suspension occurs particles tend to have the same velocity as the transporting water mass and move with it, not touching the sediment bed (Hjulström, 1939).

Clearly, the settlement velocity of particles in suspension will be influenced by their size, shape and specific gravity (Briggs, 1977; Perkins, 1974). Settlement velocities will also be affected by the specific gravity, temperature and viscosity of the medium (Perkins, 1974). Coarse sand will be concentrated near the bed whereas the finer grained constituents will be more uniformly distributed in the water column and hence usually carried further. The maximum grain size of sediment that may be held in suspension depends primarily upon the turbulence energy of the transporting medium (Reineck and Singh, 1980). This size will apparently vary greatly depending on the sedimentary environment and hydrodynamic conditions, although according to Lane (1938) it is often less than 100 μm .

In estuaries, there is a broad relationship between current velocity and the amount of sediment carried in suspension (Green, 1968; Perkins, 1974). However, the relationship is very complex and confused in its details because of the marked variability in the estuarine environment (Green, 1968; Meadows and Campbell, 1978; Perkins, 1974). Variability in the load of suspended organic matter is dependant upon wind mixing, tidal scouring and river discharge. The estuarine circulation pattern is important in

determining sediment movement (Dyer, 1979). There can often be large lateral variations in the sediment type associated with bends and junctions. Lateral variations in velocity can be large - especially in the lower part of the estuary. This can lead to sediment being transported in one direction on one side of the channel and in the opposite on the other (Dyer, 1979).

Many studies have been conducted on sediment erosion, deposition and transportation (Anderson et al., 1981; Frostick and McCave, 1979; Grant, 1981; Grant et al., 1982; McCave, 1984; Miller et al., 1977; Nickling and Eccleston, 1981; Partheniades, 1965; Southard, 1974; Settlemyre and Gardner, 1977; Steidtmann, 1982). They have been conducted on the relationship between different environmental factors (tidal action, seasonal variation), sediment characteristics (particle size distribution) and living organisms.

Frostick and McCave (1979) studied the seasonal shifts of sediment within an estuary in relation to algal growth. Their results showed an accretion of about 5 cm between April and September during algal growth, and erosion of that amount during autumn and winter when algae are dead or absent. This was due to the growth of filamentous algae (Enteromorpha) on the sediment surface probably acting as a baffle thus slowing flow near the bed and promoting deposition. Filamentous and unicellular algae can inhibit erosion both by slowing down the flow and by the secretion of mucilage (Frostick and McCave, 1979). Frostick and McCave (1979) stated that this viscous substance promotes sediment binding.

A second example is the study of Partheniades (1965) of a bluish-gray clay from Mare Island Strait in San Francisco Bay, U.S.A. This clay had high plasticity with some organic material, and its main

mineral was montmorillonite, with some illite. Its grain size composition was as follows:

- (i) clay ($<2\mu$) c. 60%;
- (ii) silt (2-50 μ) c. 40%;
- (iii) fine sand very small amounts.

Its natural water content was 110%, and it had atterberg limits of 99% (liquid limit), 44% (plastic limit), and 55% (plasticity index). Partheniades conducted experiments on control (untreated) clay and on clay that had been resuspended and then allowed to settle. This resuspension and subsequent settling provided sediments having a range of shear strengths that were less than the control untreated clay. Experiments were then conducted in a flume. He found that erosion rates were independent of the shear strength of the bed and of the concentration of suspended sediment, but that they depended strongly on the shear stress exerted by the moving water on the sediment surface - increasing rapidly after a critical value of the shear stress had been reached. He also found that for two of his clays whose shear strengths had a ratio 100:1, (1) the maximum velocity at which scouring (e.g. sediment erosion) was first observed was approximately the same, and (2) the average erosion rates at water velocities above the critical erosion velocity were of the same order of magnitude. These two findings on clays whose shear strength differ by two orders of magnitude are very remarkable.

A third example is the work of Steidtmann (1982), who studied the size-density sorting of sand-sized spherical particles during deposition in flume experiments. Steidtmann observed the motion of heavy (s.g. = 4.5) and light (s.g. = 2.5) spherical particles of a range of particle sizes, over a smooth bed and over a bed roughened by the presence of 0.35 mm diameter glass spheres. His

observations on the motion of individual heavy and light particles showed that grains smaller than bed-roughness grains (0.35 mm diameter) move continuously and have the same transport velocities regardless of density. Steidtmann (1982) also found that for grains near to and slightly larger than the bed-roughness grains, movement is intermittent and heavy particles move more slowly than light particles. Analyses of bulk sediment deposited from plane-bed transport, showed that the size and proportion of heavy particles decreased and that of light particles increased with distance transported.

(III) Theoretical aspects of sedimentation and related phenomena

1. Sedimentation theory

Tebbutt (1983) differentiates two types of particles during sedimentation.

1. Discrete particles which do not change in size, shape or mass, during settling,

and

2. flocculent particles which agglomerate during settling and thus do not have constant characteristics.

The basic theory of sedimentation (Tebbutt, 1983) assumes the presence of discrete particles. When a discrete particle is placed in a liquid of lower density it will accelerate until a limiting terminal velocity is reached, then

$$\text{gravitational force} = \text{frictional drag force}$$

$$\text{gravitational force} = (\rho_s - \rho_w)gV$$

$$\text{frictional drag force} = C_D A_C \rho_w \frac{V^2}{2}$$

The above formula leads to Stokes' Law $\left(v_s = \frac{gd^2(S_s - 1)}{18V}\right)$ which will be explained below (p/170).

The above theory cannot be applied when dealing with flocculent particles (Tebbutt, 1983). This is because of the agglomeration of floc particles which produces increased settling velocities with depth due to the formation of larger and heavier particles.

Tebbutt (1983) distinguished two types of settling based on discrete and flocculent particles respectively (Figure 23).

Class 1 settling: settlement of discrete particles in accordance with theory.

Class 2 settling: settlement of flocculent particles exhibiting increased velocity during the process. This type of settlement is as follows.

Zone settling: at a certain concentration of flocculent particles, the particles are close enough for the interparticulate forces to hold the particles fixed relative to one another so that the suspension settles as a unit.

Compressive settling: at high concentrations the particles are in contact and the weight of the particles is in part supported by the lower layers of solids.

In the case of concentrated suspensions (>2000 mg/l suspended solid), hindered settlement takes place. Here there is a significant upward displacement of water due to the settling particles and this has the effect of reducing the apparent settling velocity of the particles (Figure 24).

Hindered settling has also been described by Allen (1975) who states that settling is extremely complex especially in high concentrations and in the presence of flocculation (see also Coulson and Richardson, 1955, p. 510, for the details of hindered settling).

In a suspension of particles, the concentration of particles throughout the suspension will be initially uniform and equal. Variation in concentration within a settling suspension is explained by Allen (1975, p. 191).

Consider a small horizontal element in the suspension at a given depth. At the beginning of sedimentation the particles leaving the element are exactly balanced by the particles entering it from above. When the largest particles, initially present at the surface of the suspension leave the element, there are no similar particles entering to replace them. Hence the concentration within the element falls, as does the size of the particles.

Figure 23

Settlement of discrete and flocculent particles (from
Tebbutt, 1983, p. 109).

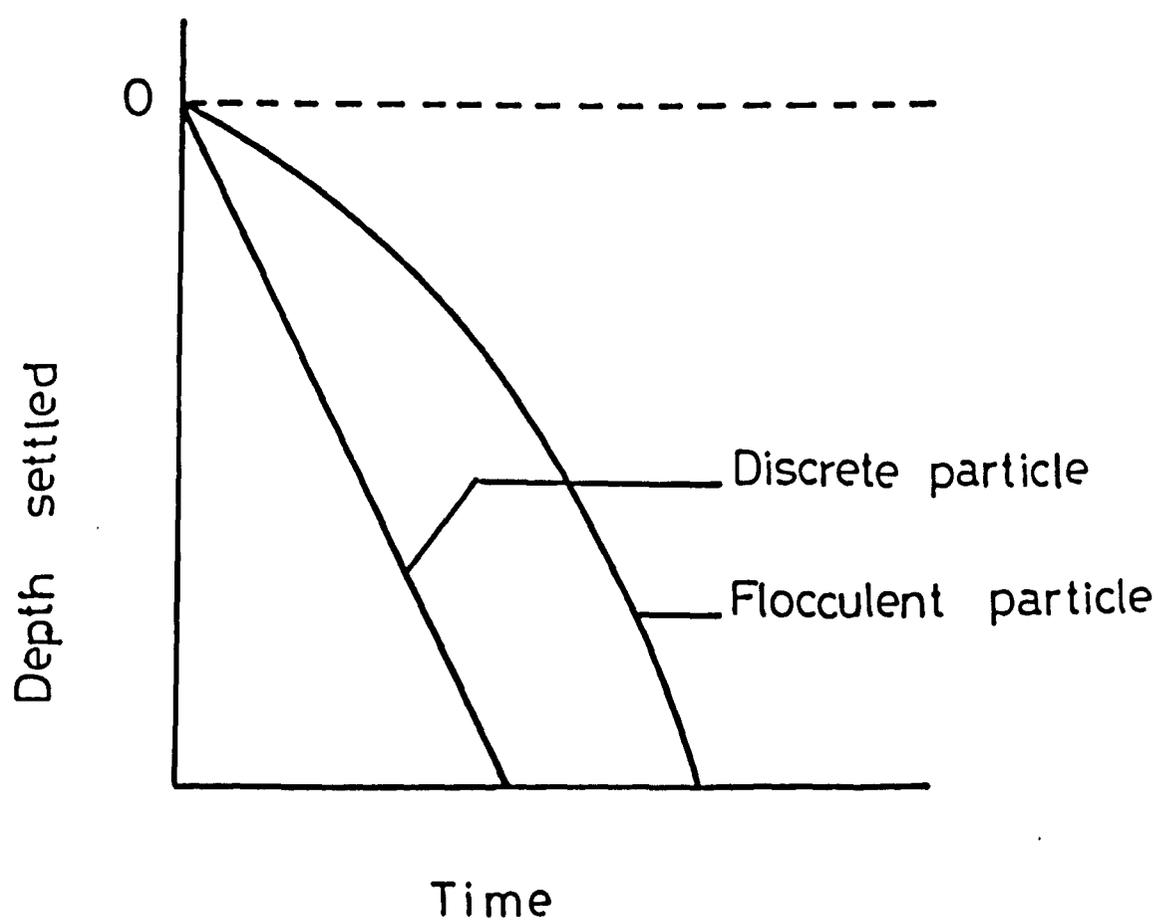
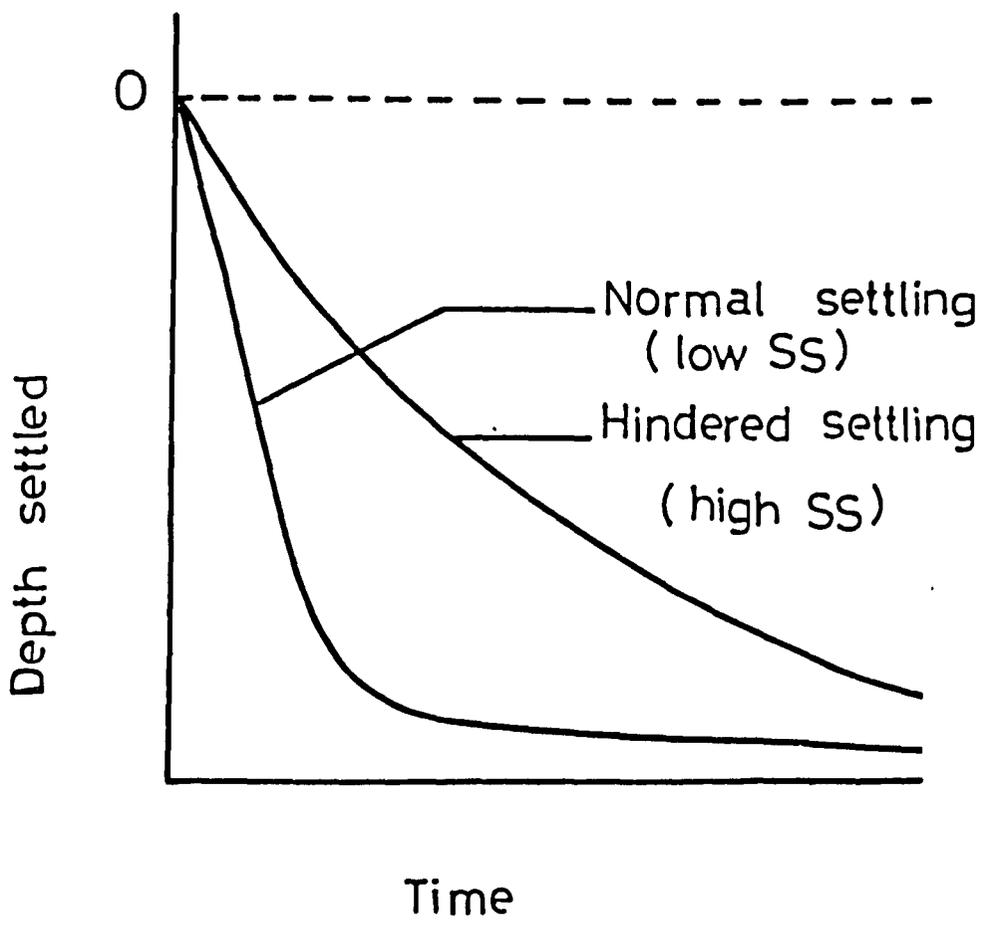


Figure 24

Hindered settling (from Tebbutt, 1983, p. 110).



2. Stokes' Law

The settling velocities of fine particles (i.e. silt and clay) are usually computed from the settling law developed by G.G. Stokes in 1851 (Galehouse, 1971; Massey, 1979; Smith, 1981). Stokes' Law pertains to the terminal fall velocity of a sphere in a fluid and is as follows (Galehouse, 1971).

VRF (the viscous resistance to fall of a sphere in a fluid) =

$$6\pi r \mu v.$$

Where r = radius of the sphere in cm,

μ = viscosity of the fluid in dyne-sec/cm² (poises),

v = fall velocity in cm/sec,

and NDF (the net downward force on a sphere in a fluid) = the force of gravity on the sphere minus buoyant force of the fluid.

$$\text{NDF} = \frac{4}{3} (\pi r^3 d_s g) - \frac{4}{3} (\pi r^3 d_f g)$$

Where r = radius of the sphere in cm,

d_s = density of the sphere in gm/cm³,

d_f = density of the fluid in gm/cm³,

g = acceleration due to gravity.

However, the terminal fall velocity reached when

$$\text{VRF} = \text{NDF}$$

that is, when

$$6\pi r \mu v = \frac{4}{3} (\pi r^3 d_s g) - \frac{4}{3} (\pi r^3 d_f g)$$

or when

$$v = \frac{2(d_s - d_f) g r^2}{9\mu}$$

which is Stokes' Law, where v is now the terminal fall velocity of the sphere.

Stokes' Law as used in sedimentation analysis at a particular temperature is commonly simplified to

$$V = CD^2$$

where C is a constant equalling

$$C = \frac{(d_s - d_f)g}{18\mu}$$

and d_s = the density of the sediment particles (= 2.65 gm/cm³ for quartz),

d_f = the density of the liquid through which the particles are falling,

g = 980 cm/sec²,

μ = the viscosity of the liquid at the particular temperature

and D = the diameter of the sphere in cm.

If the settling velocity is known for a particular temperature, D can be calculated as

$$D = \frac{\sqrt{V}}{\sqrt{C}}$$

In summary, Stokes' Law or Wadell's Formula (1934) (see below p. 171) are restricted to the range of silt and clay sized particle.

3. Limitations of Stokes' Law (Galehouse, 1971)

1. Particles must have reached terminal fall velocity. For particles within the range of Stokes' Law, the terminal fall velocity is reached almost instantaneously.

2. Particles must be rigid. All particles analysed sedimentologically fulfil this condition.

3. Particles must be smooth. Most particles analysed sedimentologically are not smooth. Arnold (1911) has shown that within the size range of applicability of Stokes' Law, grains with irregular

surfaces do not have any appreciable difference in settling velocity from smooth grains.

4. No slippage or shear should take place between the particle and the fluid. This depends on the wettability of the particle in the fluid, and the condition is fulfilled when water is used as the fluid.

5. The fluid must be of infinite extent in relation to the particles. A particle settling near the wall of a container will have its settling velocity decreased by an amount dependent on the nearness of the wall and the size of the particle (Krumbein and Pettijohn, 1938, Figure 21, p. 99). In the size range of Stokes' Law, the wall effects are negligible if the sedimentation vessel is greater than 4 cm in diameter. Most 1000-ml graduated cylinders used in the pipette analysis of silt and clay are larger than this.

6. Particle concentration must be less than 1% (w/v).

7. Particles must be greater than 0.5μ in diameter. Very small particles are affected by Brownian movement of the molecules of the fluid. This keeps the particles from falling in a straight line, and consequently the resistance to fall is no longer only a function of the particle size and the velocity of the fluid.

8. Particles must not be greater than 50μ in diameter. The upper limit to the size of particles settling according to Stokes' Law is a function of temperature and Reynold's number of the fluid and the density of the particles. Above this limit there is turbulence during settling. However, Rubey (1933) shows that observed settling velocity differs little from the theoretically determined Stokes' values up to about 140μ (Galehouse, 1971).

9. Particles must be spheres. In nature, practically no particles are perfect spheres. Wadell (1934), using the same basic assumptions as Stokes, developed a settling velocity formula that takes particle shape into consideration. Wadell used a particle shape between that of a sphere and a disk which is much closer to the average shape in nature than is a sphere. In essence, Wadell's formula reduces the settling velocity determined by Stokes' Law. To convert any Stokes' value to a Wadell value, multiply by 0.64.

4. Methods of sedimentation size analysis

There are several different methods of sedimentation size analysis (Allen, 1975; Dyer, 1979). These methods are summarised in Appendix II, pp. 514-516.

5. Laminar and turbulent flow of water

There are two distinctly different types of fluid flow (Fox, 1974; Douglas et al., 1979; Massey, 1979; Mironer, 1979; Perkins, 1974; Walshaw and Jobson, 1972). These two types are (a) laminar flow, and (b) turbulent flow.

(a) Laminar flow

This occurs in water currents of low velocity, where the particles move in a parallel way, without cross currents (Perkins, 1974). It is a non-turbulent flow of a fluid in which parallel layers have different relative velocities. A laminar flow moves as if it were composed of very thin sheets, or laminae of fluid (Massey, 1979) each gliding smoothly over its neighbour (Mironer, 1979). There is almost no macroscopic mixing or exchange of fluid between neighbouring sheets. Any small disturbances in the flow are dampened by the internal friction or viscous resistance of the flow (Mironer, 1979).

(b) Turbulent flow

This occurs in water in which the velocity at any point varies rapidly in an irregular manner. In some cases turbulent flow occurs as a movement against the net direction of flow of the current (Perkins, 1974). This is the case in rivers and estuaries. It is a disorderly flow, with irregular high frequency fluctuations of velocity superimposed on the main motion (Massey, 1979; Mironer, 1979). Small but macroscopic pieces of fluid are transported by the turbulent motion in all directions and mix with other parts of the flow. Disturbances in a turbulent flow are not usually dampened but grow and further contribute to the random fluctuations (Mironer, 1979).

6. Reynolds' demonstration of laminar and turbulent flow

Both engineers and mathematicians have contributed to the study of fluid motion, and in recent years there has been a marked tendency to use mathematical methods and theories to follow and measure the motion of fluids.

Reynolds (1883) was the first to demonstrate the two types of flow experimentally. Reynolds conducted three classic experiments in which he observed the difference between laminar and turbulent water motion. In the first, he noted the damping effect of oil on waves in a small pond. In the second, he allowed water to flow through tubes coming from a large glass tank, and allowed streaks of highly coloured water to enter the tubes with the clear water. In the third, he placed a layer of carbon disulphide under a layer of water in a long horizontal tube. These two liquids are immiscible, so Reynolds was able to observe the development of small waves between the two liquids as the tube was inclined.

His results showed that:

- (a) there is a critical velocity above which flow becomes unstable (turbulent flow);
- (b) the instability comes on gradually and does not depend on the magnitude of the disturbances; in other words, the eddies first make their appearance as small disturbances, and then increase gradually with velocity.

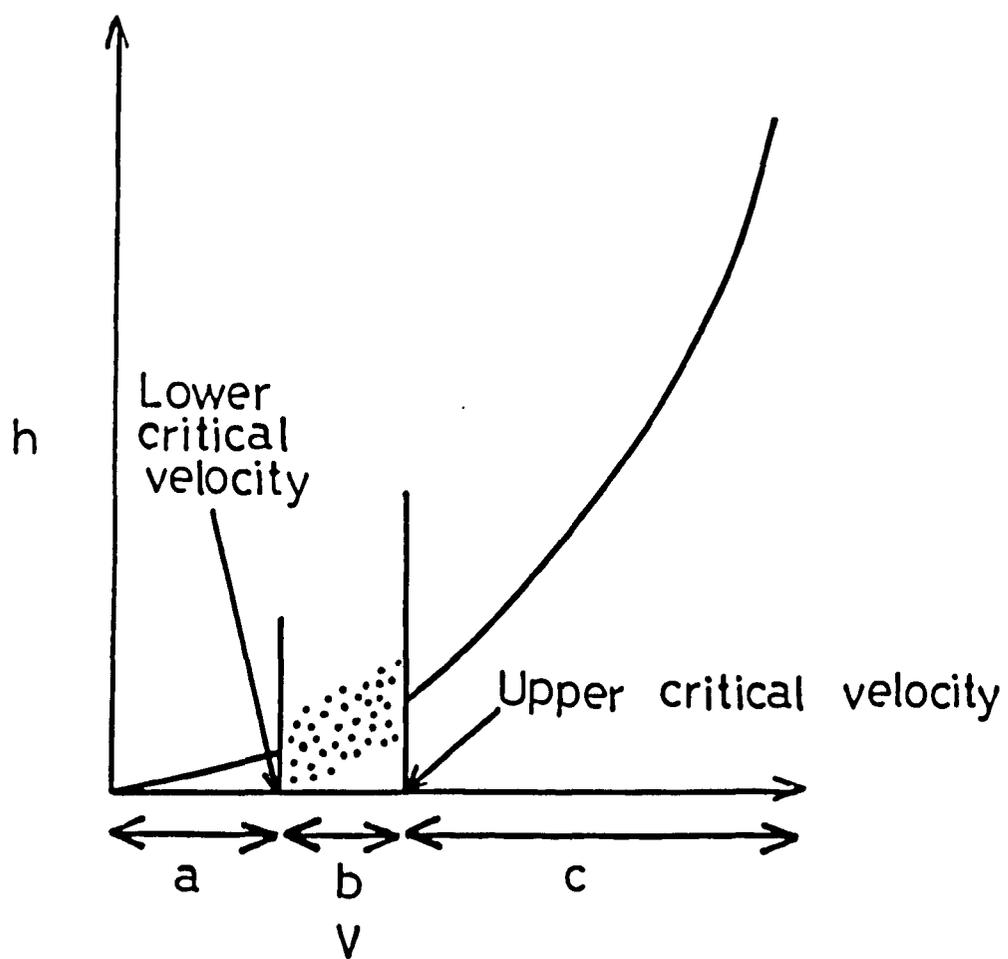
7. Lower and upper critical velocity and laminar and turbulent flow

The results of Reynolds' experiments have been graphically represented by Fox (1974, p. 176) and Massey (1979, p. 133). Below a lower critical velocity, flow is laminar and above an upper critical velocity motion is randomised and irregular - i.e. turbulent (Figure 25). As the flow changes from laminar to turbulent it passes through a transition phase as it moves from the lower to the upper critical velocity. The laminar flow becomes increasingly unstable and turbulence fluctuations begin to develop until full turbulence occurs.

Under natural conditions, flow of water is either laminar or turbulent, but turbulent flow is by far the most common (Mironer, 1979).

Figure 25

Lower and upper critical velocities, and laminar and turbulent flow (from Fox, 1974, p. 177). Diagram illustrating the relationship between velocity (V) of liquid flowing through a pipe and the pressure difference (h) between the ends of the pipe. a = laminar flow, b = transitional phase, c = turbulent flow.



(IV) Recent work on sediments and sedimentation

1. Sediments

Interest in the study of the depositional environment of sediments has increased enormously over the past fifteen years. A large number of research studies have been carried out on sediments and their components, on the effect of environmental factors, and on the physical and chemical properties of sediments and their dynamics.

Daubr e (1879) was probably the first to conduct experiments on sediments and studied the resistance of sand grains to abrasion during transportation (Russell and Taylor, 1937). Since then a large number of studies have been conducted on sediment grains and their characteristics.

Studies have been carried out on the shape, morphology, size and distribution of sediment grains (Bagnold and Barndroff-Nielson, 1980; Barret, 1980; Burger, 1976; Flood, 1981; Wang et al., 1982; Winkelmoen, 1982). Another group of research workers have studied relationships between grain size and water flow (Allen, 1977; Boersma and Terwindt, 1981; Brayshaw et al., 1983; Channon and Hamilton, 1976; Einsele, 1977; Ghosh et al., 1981; Hedges and Parker, 1976; Lambiase, 1980; Langhorne, 1982; Mantz, 1978; Reice, 1977; Sengupta, 1979).

A third group of research workers studied particle size and its relation to settling velocity (Collins and Rigler, 1982; Gibbs et al., 1971; Hallermeier, 1981; Hulsey, 1961; Kajihara, 1971; Kuenen, 1968).

Many studies have been quoted previously in this introduction which are related to the characteristics and behaviour of sediment particles during deposition, erosion and transportation. There are

also a large number of studies on the relationship between organisms and their sedimentary environment. Organism-Sediment relationships will be described in detail in the following section (i.e. Section III).

2. Sedimentation

Sedimentation has been studied by a number of research workers. These studies were carried out on different aspects of sedimentation and can be classified as follows. Studies have been conducted on the relationships between particle size, flocculation, distribution and the effects of these factors on sedimentation (Krank, 1975; McCave, 1970; Visher, 1969). Other studies were related to chemical and physical conditions and some aspects of the rate and nature of sedimentation (Carling, 1982; Moore, 1931; Peirce and Williams, 1966). A third group of studies were carried out on the dynamics and on the temporal, and spatial nature of sedimentation (Gasith, 1976; Jopling, 1964; Lowe, 1976; Richardson and Zaki, 1954).

Jopling (1964) considered Otto's (1938) concept of a sedimentation unit. Otto (1938) defined the sedimentation unit as that thickness of sediment which is deposited under essentially constant physical conditions. The finer subdivisions of this unit were defined as laminations. Jopling (1964) conducted laboratory experiments that demonstrated that a well defined bedded structure can develop under-state conditions of flow (velocity) and sediment transport. Furthermore, the experimental evidence suggests that a small change in conditions may initiate the deposition of a group of laminae rather than single lamina. Based on his experimental evidence Jopling stated that the words "essentially constant" used by Otto in his definition of the sedimentation unit must be interpreted with caution in the light of present knowledge. Jopling also stated that a well-defined lamination can be formed under an apparently wide range of

flow conditions, including the special case of uniform flow conditions.

Richardson and Zaki (1954) studied sedimentation and fluidisation. In sedimentation, suspended particles fall under the influence of gravity in a stationary fluid, while in fluidisation, the particles are kept in suspension by an upward flow of liquid. They described the theory behind both processes and then correlated this with their experimental results.

Gasith (1976) studied seston dynamics and tripton sedimentation in the pelagic zone of a shallow eutrophic freshwater lake (Wingra) in Wisconsin. Gasith defines tripton as organic detritus consisting of plant and animal remains with their associated fauna and flora of grazers and decomposers, and seston as being composed primarily of resuspended bottom sediment and living algae and zooplankton. Sedimentation of tripton was closely related to the dynamics of seston with a lag of two to four weeks. Water turbulence, as well as differences in phytoplankton associations and phytoplankton-zooplankton interactions, were suggested as important factors influencing tripton sedimentation. Gasith (1976) estimated that his sedimentation process could account for the removal of more than 40% of the annual macrophyte and phytoplankton production. He also estimated that 70% of the settling detritus decomposed annually. Consequently, only a small fraction of the tripton was involved in the long term accretion of lake sediment.

(V) Aspects of sedimentation studied in Sections II and III of the thesis

Most of the inorganic and organic material which forms sediments in estuaries is carried there by the river and originates from land erosion. Mineral grains, such as quartz, sediment through the action of gravity, while organic materials become precipitated when they meet the increased salt concentration in an estuary. This is the way in

which large estuarine mud-flats are formed and maintained. Sediment on these mud-flats is continuously resuspended by tidal and wave action and then sinks again to the bottom.

The work I describe in Sections II and III is concerned with this secondary sedimentation process of materials which has been locally resuspended. Section II deals with some of the physical and chemical effects on this sedimentation, and Section III describes how secretions produced by benthic organisms at the surface of the sediment affect sedimentation of particles once they have been resuspended.

(VI) Aims of the work presented in this section

The sedimentary processes and water flow characteristics occurring in estuaries indicate that the intertidal zone is a very complex habitat, and there are wide fluctuations in a range of environmental variables that are likely to affect sediments. These variables may include salinity, temperature, intertidal drying (dehydration) and organic coatings on sediment particles. The main aim of the research presented in this section was to test the effect of these four variables on sedimentation. The following four short subsections review the range of variation that can occur in the four variables.

1. Temperature fluctuations

Wide rapid changes in temperature can occur in the intertidal zone. When the tide recedes, sunshine can produce a very high temperature on the exposed area. Temperature in the intertidal zone fluctuates from 0°C during a cold winter to 30°C or 40°C during a hot summer (Meadows and Campbell, 1972, 1978).

2. Salinity fluctuations

Wide ranges of salinities can occur in the intertidal area. High salinities can be produced by evaporation of water during warm weather.

Conversely, rainfall or freshwater run-off can produce very low salinities. Salinities in the intertidal zone can vary from $< 0.5^{\circ}/\text{oo}$ to above $35^{\circ}/\text{oo}$ or $37^{\circ}/\text{oo}$ in Britain (see Section I, Discussion, p. 130 for references).

3. Dehydration

Intertidal sediments are subjected to periods of exposure and drying during low tide, but are covered by water during high tide. Although a water film is generally present between sediment particles during low tide because of capillary attraction (Green, 1968), surface sediment can be dried by the action of sun and wind. This is especially true during the summer months.

Now surface sediment will be disturbed by the incoming tide, and some of this will be in suspension. This suspended material will therefore contain some sediment grains which were dry or partially dry. The sedimentation of suspended particles which have been previously dried may be different than the sedimentation of particles which have remained wet.

4. Organic coatings on sediment particles

Sediment particles are covered by a film of different types of microorganisms and organic materials. The occurrence of this film or layer has been recorded in a number of studies (Anderson and Meadows, 1969, 1978; Anderson, Boonruang and Meadows, 1981; Gray, 1967a; Meadows and Anderson, 1966, 1968). Treatments which kill, inactivate, or remove microorganisms render sands unattractive to benthic invertebrates (Gray, 1966, 1967; Gray and Johnson, 1970; Meadows, 1964; Wieser, 1956; Wilson, 1955).

Removal or partial removal of the surface film of particles may affect sedimentation. This can occur under natural conditions: the

surfaces of sediment particles may be cleaned by factors such as rainfall (Anderson and Meadows, 1978), the feeding activities of benthic animals (Fenchel et al., 1975; Hylleberg, Kristensen, 1972), and possibly by direct microbial degradation (Fenchel, 1970, 1972).

SECTION II

Materials and Methods(I) Collection, maintenance and preparation of sediments

Langbank, Ardmore and Rockware sediments were used in the experiments reported in this section (see Section I, p.32-33).

Samples containing animals from both beaches were transported in buckets to an aquarium. Langbank and Ardmore sediments were carefully sieved for experiments by wet sieving through a 710 μm sieve (Endecotts Ltd, London, England - Laboratory Test Sieve) which removed all macrobenthic animals.

All three sediments were maintained in aerated synthetic sea water (prepared from Natura Sea Salt, supplied by Philip Harris Biological Ltd, Oldmixon, Weston-Super-Mare, Avon). The sea water was made up with tap water (local tap water is very soft and almost as pure as distilled water).

The remainder of the materials and methods in this section is divided into three parts. The first (Part II) gives the preliminary experiments to determine the most suitable sampling time and volume, and the definitive method which was used in measuring particles' sedimentation in all experiments. The second (Part III) tests how the removal of different amounts of organic materials from sediment particles influences their sedimentation, the use of ashing and acid-cleaning. The third (Part IV) gives the effect of three different temperatures, three different salinities, and wet and dry sediment on sedimentation.

(II) Sedimentation experiments

This part is divided into two divisions. The first (1) gives a quick method for obtaining accurate dry weights of sediment for

sedimentation experiments. The second (2) records the preliminary experiment testing the sampling time, volume, and from these the definitive method for measuring sedimentation.

1. Determination of a quick method for obtaining accurate dry weights of sediments for sedimentation experiments

The design and execution of the sedimentation experiments demanded that a quick method should be used to obtain aliquots of sediment for the experiments. It was decided to use a volume measure of sediment. This volume method was calibrated as follows.

Twenty replicates each of Langbank, Ardmore and Rockware sediments were used. A sediment sample was placed in a small glass petri dish (diameter: 5.3 cm) and levelled. Levelled samples were transferred to a small metal foil box of known weight. The sediment sample was then weighed and dried in an oven at 60°C for 48 hours. The sample was placed in a desiccator to cool and reweighed after drying. The results are shown in Table 19, with their means and standard deviations.

The data were analysed by two one-way anovars. The first compared the wet weights between the three sediments and the second compared the dry weights between the three sediments. The results of these analyses (Table 20) show that there were significant differences between the wet weights but not between the dry weights. Breakdown one X two one-way anovars confirmed these conclusions but are not quoted.

Since there was no difference between the dry weights of the three sediments in the above experiments, the above experimental method was used in all the subsequent sedimentation experiments expressing all results on a dry weight basis.

The same glass petri dish (internal diameter = 5.3 cm, volume = 19.86 cm³) was used in all the experiments from here onwards - filling it with wet sediment and levelling on each use. This meant that between 42 and 43 g of dry sediment was always used. The mean and standard deviation of the added weight of dried sediment was always taken as 42.61 ± 1.301 g, which is the mean and standard deviation of all the 60 dry weights in Table 19.

TABLE 19. Wet and dry weight of Langbank (LBM), Ardmore (ARD) and Rockware (RWS) sediments, and their means and standard deviations obtained from twenty replicates in each case.

| R | LBM (g) | | ARD (g) | | RWS (g) | |
|--------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | Wet | Dry | Wet | Dry | Wet | Dry |
| 1 | 57.41 | 42.79 | 54.20 | 41.45 | 53.01 | 42.90 |
| 2 | 58.53 | 43.89 | 56.42 | 43.94 | 53.28 | 42.00 |
| 3 | 58.68 | 44.81 | 53.61 | 41.18 | 52.24 | 42.35 |
| 4 | 59.11 | 45.12 | 55.87 | 42.33 | 52.18 | 42.29 |
| 5 | 54.77 | 40.85 | 55.25 | 41.18 | 53.49 | 42.79 |
| 6 | 58.50 | 45.01 | 53.83 | 41.36 | 53.28 | 43.63 |
| 7 | 57.39 | 41.69 | 53.95 | 44.88 | 53.31 | 43.36 |
| 8 | 56.41 | 42.53 | 58.51 | 44.54 | 52.25 | 42.56 |
| 9 | 58.08 | 43.96 | 56.02 | 42.89 | 51.46 | 41.07 |
| 10 | 54.50 | 40.45 | 57.41 | 43.22 | 51.59 | 41.48 |
| 11 | 56.66 | 42.12 | 54.54 | 40.99 | 51.73 | 42.03 |
| 12 | 55.67 | 42.10 | 55.72 | 42.49 | 50.61 | 40.57 |
| 13 | 55.69 | 41.61 | 55.70 | 42.25 | 51.30 | 40.82 |
| 14 | 59.27 | 44.70 | 58.64 | 43.98 | 53.47 | 42.67 |
| 15 | 56.70 | 43.48 | 56.13 | 42.14 | 53.89 | 42.71 |
| 16 | 54.07 | 40.89 | 57.22 | 43.42 | 51.28 | 41.17 |
| 17 | 58.50 | 44.34 | 54.78 | 41.55 | 53.01 | 41.77 |
| 18 | 58.46 | 44.17 | 57.38 | 44.21 | 56.26 | 44.61 |
| 19 | 52.56 | 39.96 | 56.84 | 42.85 | 54.29 | 42.72 |
| 20 | 57.12 | 42.27 | 54.97 | 41.99 | 53.73 | 43.33 |
| Means + s.d. | 56.90 + 1.868 | 42.84 + 1.624 | 55.85 + 1.494 | 42.65 + 1.210 | 52.78 + 1.313 | 42.34 + 1.013 |

TABLE 20. One-way analysis of variance for the three types of sediments (wet and dry): each anovar with three levels (three sediment types).

| Sediment type | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|---------------|---------|----------------|--------------|-----|---------|------------------------|
| Wet | Main | 183.3 | 91.66 | 2 | 36.95 | P < 0.001 ***** |
| | Error | 141.4 | 2.481 | 57 | | |
| | Total | 324.7 | | 59 | | |
| Dry | Main | 2.492 | 1.246 | 2 | 0.7291 | 0.50 > P > 0.25 |
| | Error | 97.42 | 1.709 | 57 | | |
| | Total | 99.92 | | 59 | | |

2. Preliminary experiments to decide the sampling time, volume and the definitive method for measuring sedimentation

A number of preliminary experiments were conducted to determine the sampling time and volume required to measure particle sedimentation. These preliminary experiments were conducted using Langbank and Rockware sediments only. The following method was used.

A levelled petri dish full of wet sediment was added to 500 ml of synthetic sea water in a 500 ml stoppered glass measuring cylinder (internal diameter: 4.75 cm). In all experiments the cylinder was inverted three times by hand before taking each sample. Pipette samples were always taken at 10 cm depth below the water level, and from the centre of the cylinder. Each sample was pipetted into a separate metal foil box, dried at 60°C for 24 hours and weighed. Allowance was made for the weight of salt in the solution by drying twenty replicate samples of sea water, and subtracting the mean of these readings from all the weights in the 100% sea water experiments, and half the mean from all the weights in the 50% sea water experiments. No correction was made to the weights in the distilled water experiments.

(a) Experiments to decide the sampling time

Five replicate 2 ml pipette samples were removed at 5 minutes, 10 minutes, 20 minutes, 40 minutes, 60 minutes and 24 hours. Samples were dried, and allowance was made for the salt in the solution as above. All of these times proved to be too long, because virtually no sediment remained in suspension even after 5 minutes.

Another experiment was therefore designed in which 5 seconds, 10 seconds, 20 seconds, 40 seconds, 60 seconds and 120 seconds, were tested. The results of this experiment are shown in Table 21, and it is clear that these times give suitable weights for definitive

TABLE 21. Experiment assessing best time for sediment sampling in the sedimentation experiments. Means and standard deviations of suspended weights (mg/ml) of Langbank (LBM) and Rockware (RWS) sediments, removed with successive equal volume of liquid at different times (5, 10, 20, 40, 60 and 120 seconds).

| Sediment | Time intervals (secs) | | | | | |
|----------|------------------------|------------------------|------------------------|-------------------------|---------------------------|-------------------------|
| | 5 | 10 | 20 | 40 | 60 | 120 |
| LBM | 2.808 + 0.1627 | 2.025 + 0.06614 | 1.192 + 0.1702 | 0.4833 + 0.06292 | 0.3667 + 0.01443 | 0.03333 + 0.01443 |
| RWS | 0.5750 + 0.02500 | 0.4750 + 0.05000 | 0.4583 + 0.02887 | 0.05500 + 0.00500 | 0.006667 + 0.002887 | 0.005000 + 0 |

experimentation. These times were therefore used in all further experiments except where stated.

The variation of suspended weight with sediment type and sampling time was analysed for statistical significance using a two-way analysis of variance. The results of this analysis are shown in Appendix II, Table 1, p. 458). Highly significant interaction was found ($P < 0.001$). Breakdown one-way analysis of variance were therefore carried out. Table 22 shows the results of these analyses. A highly significant variation of suspended weight with sampling time was found with both types of sediment (both $P < 0.001$), as is to be expected.

TABLE 22. Two one-way analysis of variance of the suspended weight of Langbank (LBM) and Rockware (RWS) sediments, removed with successive equal volume of liquid at different time intervals (5, 10, 20, 40, 60, 120 secs), which was measured to determine the sampling time.

| Sediment | Factors | Sum of squares | Means squares | D.F. | F-ratio | Probability |
|----------|---------|----------------|---------------|------|---------|------------------------|
| LBM | Main | 17.47 | 3.493 | 5 | 326.7 | P < 0.001 ***** |
| | Error | 0.1283 | 0.010694 | 12 | | |
| | Total | 17.60 | | 17 | | |
| RWS | Main | 1.068 | 0.2136 | 5 | 321.05 | P < 0.001 ***** |
| | Error | 0.007983 | 0.0006653 | 12 | | |
| | Total | 1.076 | | 17 | | |

(b) Experiments to decide the sampling volume

Twenty replicate samples of 1, 2, 4, 6 and 8 ml were removed at 5 seconds after inversion. Samples were dried and allowance was made for the salt content in the solution. The results of the volume experiments (Figure 26), show that the largest weight of sediment (mg/ml) was obtained in the 2 ml sample. A sample size of 2 ml (taken at 10 cm depth) was therefore used in all the definitive experiments.

The variation of suspended weight with sediment type and sampling volume was analysed for statistical significance using a two-way analysis of variance. The results of this analysis are shown in Appendix II, Table 2, p. 459. Significant interaction was found ($0.025 > P > 0.01$). Breakdown one-way analyses of variance were therefore carried out. Table 23 shows the results of these analyses. A highly significant variation of suspended weight with sampling volume was found with both types of sediments (both $P < 0.001$). A series of breakdown one-way analyses of variance were carried out for each sediment type, to compare the suspended weights found at each sampling volume. Tables 24, 25, 26 and 27 show the results of these analyses. With Langbank sediment (Tables 24 and 25), highly significant differences were apparent in weight between each sampling volume ($P < 0.001$), except between 4 ml and 6 ml ($0.75 > P > 0.50$), 4 ml and 8 ml ($0.50 > P > 0.25$) and between 6 ml and 8 ml ($0.50 > P > 0.25$). With Rockware sediment (Tables 26 and 27), highly significant differences were apparent in suspended weight between each sampling volume ($P < 0.001$), except between 1 ml and 4 ml ($0.10 > P > 0.05$), 1 ml and 6 ml ($0.005 > P > 0.001$) and between 4 ml and 6 ml ($0.50 > P > 0.25$).

Figure 26

Experiment assessing the best volume for sediment sampling in the sedimentation experiments. Mean suspended weight of Langbank (LBM) and Rockware (RWS) sediments taken by different sampling volumes (1, 2, 4, 6 and 8 ml). Standard deviations are too small to plot.

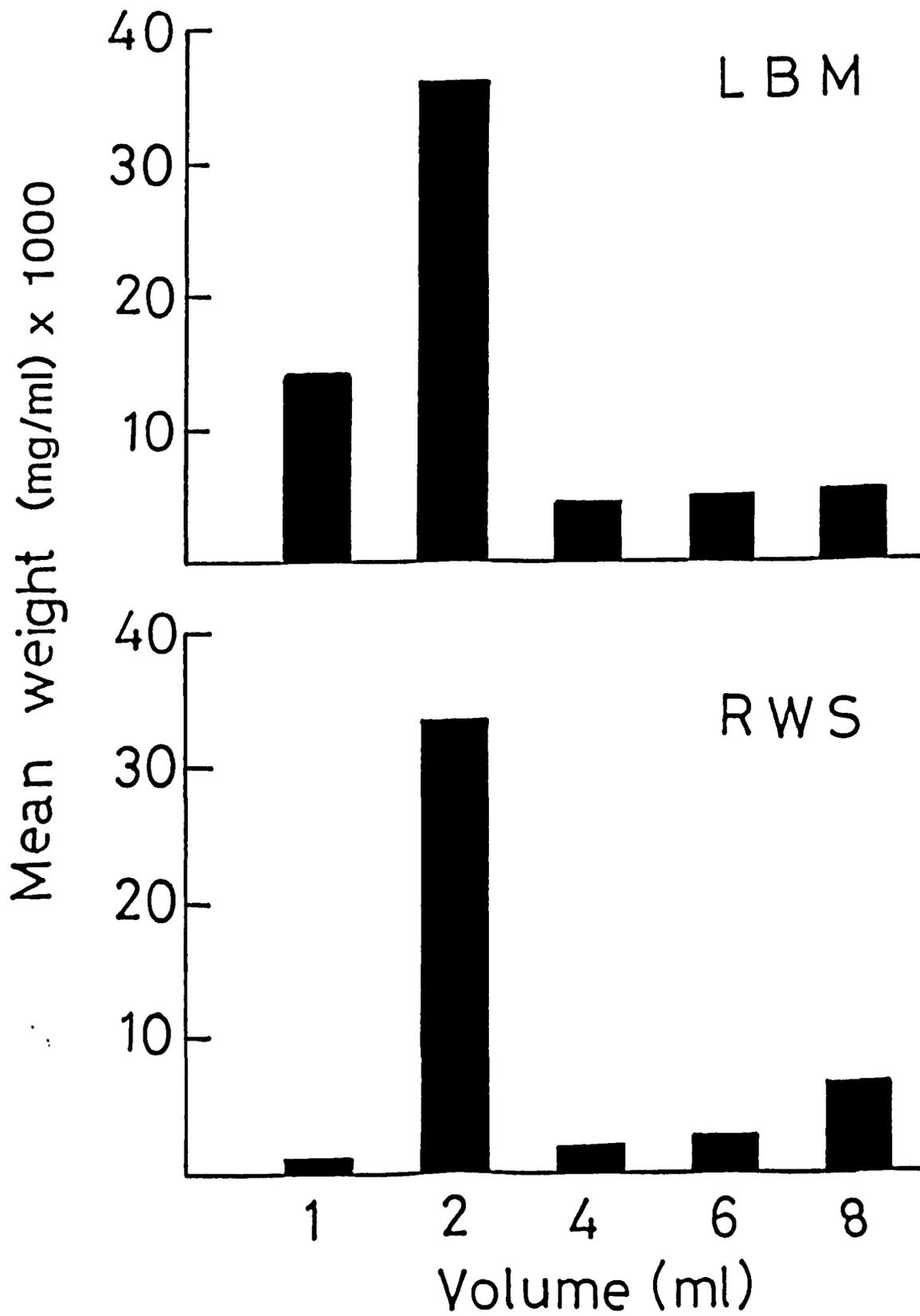


TABLE 23. Two one-way analyses of variance of the suspended weights of Langbank (LBM) and Rockware (RWS) sediments, removed with different volume of liquid at (1, 2, 4, 6 and 8 ml) at 5 seconds after inversion to determine the sampling volume.

| Sediment | Factors | Sum of squares | Means squares | D.F | F-ratio | Probability |
|----------|---------|----------------|---------------|-----|---------|-------------|
| LBM | Main | 14761.3 | 3690.3 | 4 | 37.73 | $P < 0.001$ |
| | Error | 9293.0 | 97.82 | 95 | | |
| | Total | 24054.3 | | 99 | | ***** |
| RWS | Main | 15254.1 | 3813.5 | 4 | 83.44 | $P < 0.001$ |
| | Error | 4341.8 | 45.70 | 95 | | |
| | Total | 19595.9 | | 99 | | ***** |

TABLE 24. A series of one-way breakdown analyses with two levels each, comparing the suspended weights of Langbank sediment removed with different volumes of liquid at 5 seconds after inversion to determine the sampling volume (1 = 1 ml, 2 = 2 ml, 3 = 4 ml, 4 = 6 ml and 5 = 8 ml).

| Groups compared | Factors | Sum of squares | Means squares | D.F | F-ratio | Probability |
|-----------------|---------|----------------|---------------|-----|---------|------------------------|
| 1 x 2 | Main | 4974.7 | 4974.7 | 1 | 22.52 | P < 0.001 ***** |
| | Error | 8392.5 | 220.9 | 38 | | |
| | Total | 13367.2 | | 39 | | |
| 1 x 3 | Main | 947.7 | 947.7 | 1 | 26.00 | P < 0.001 ***** |
| | Error | 1384.8 | 36.44 | 38 | | |
| | Total | 2332.5 | | 39 | | |
| 1 x 4 | Main | 807.4 | 807.4 | 1 | 27.68 | P < 0.001 ***** |
| | Error | 1108.5 | 29.17 | 38 | | |
| | Total | 1915.9 | | 39 | | |
| 1 x 5 | Main | 696.2 | 696.2 | 1 | 28.36 | P < 0.001 ***** |
| | Error | 932.9 | 24.55 | 38 | | |
| | Total | 1629.1 | | 39 | | |
| 2 x 3 | Main | 10264.8 | 10264.8 | 1 | 48.19 | P < 0.001 ***** |
| | Error | 8093.5 | 213.0 | 38 | | |
| | Total | 18358.3 | | 39 | | |

TABLE 25. A series of one-way breakdown analyses with two levels each, comparing the suspended weights of Langbank sediment removed with different volumes of liquid at 5 seconds after inversion to determine the sampling volume (2 = 2 ml, 3 = 4 ml, 4 = 6 ml and 5 = 8 ml).

| Groups compared | Factors | Sum of squares | Means squares | D.F | F-ratio | Probability |
|-----------------|---------|----------------|---------------|-----|---------|------------------------|
| 2 x 4 | Main | 9790.3 | 9790.3 | 1 | 47.59 | P < 0.001 ***** |
| | Error | 7817.2 | 205.7 | 38 | | |
| | Total | 17607.5 | | 39 | | |
| 2 x 5 | Main | 9393.1 | 9393.1 | 1 | 46.71 | P < 0.001 ***** |
| | Error | 7641.6 | 201.1 | 38 | | |
| | Total | 17034.7 | | 39 | | |
| 3 x 4 | Main | 5.956 | 5.956 | 1 | 0.2791 | 0.75 > P > 0.50 |
| | Error | 811.0 | 21.34 | 38 | | |
| | Total | 817.0 | | 39 | | |
| 3 x 5 | Main | 19.34 | 19.34 | 1 | 1.159 | 0.50 > P > 0.25 |
| | Error | 633.9 | 16.68 | 38 | | |
| | Total | 653.3 | | 39 | | |
| 4 x 5 | Main | 4.113 | 4.113 | 1 | 0.4372 | 0.50 > P > 0.25 |
| | Error | 357.5 | 9.409 | 38 | | |
| | Total | 361.6 | | 39 | | |

TABLE 26. A series of one-way breakdown analyses with two levels each, comparing the suspended weights of Rockware sediment removed with different volumes of liquid at 5 seconds after inversion to determine the sampling volume (1 = 1 ml, 2 = 2 ml, 3 = 4 ml, 4 = 6 ml and 5 = 8 ml).

| Groups compared | Factors | Sum of squares | Means squares | D.F | F-ratio | Probability |
|-----------------|---------|----------------|---------------|-----|---------|-------------------------------|
| 1 x 2 | Main | 10653.7 | 10653.7 | 1 | 100.1 | P < 0.001 ***** |
| | Error | 4044.8 | 106.4 | 38 | | |
| | Total | 14698.5 | | 39 | | |
| 1 x 3 | Main | 10.59 | 10.59 | 1 | 4.051 | 0.10 > P > 0.05 |
| | Error | 99.32 | 2.614 | 38 | | |
| | Total | 109.9 | | 39 | | |
| 1 x 4 | Main | 32.58 | 32.58 | 1 | 10.89 | 0.005 > P > 0.001 **** |
| | Error | 113.7 | 2.993 | 38 | | |
| | Total | 146.3 | | 39 | | |
| 1 x 5 | Main | 334.3 | 334.3 | 1 | 131.7 | P < 0.001 ***** |
| | Error | 96.46 | 2.538 | 38 | | |
| | Total | 450.8 | | 39 | | |
| 2 x 3 | Main | 9992.6 | 9992.6 | 1 | 91.81 | P < 0.001 ***** |
| | Error | 4135.8 | 108.8 | 38 | | |
| | Total | 14128.4 | | 39 | | |

TABLE 27. A series of one-way breakdown analyses with two levels each, comparing the suspended weights of Rockware sediment removed with different volumes of liquid at 5 seconds after inversion to determine the sampling volume (2 = 2 ml, 3 = 4 ml, 4 = 6 ml and 5 = 8 ml).

| Groups compared | Factors | Sum of squares | Means squares | D.F | F-ratio | Probability |
|-----------------|---------|----------------|---------------|-----|---------|------------------------|
| 2 x 4 | Main | 9507.9 | 9507.9 | 1 | 87.06 | P < 0.001 ***** |
| | Error | 4150.2 | 109.2 | 38 | | |
| | Total | 13658.1 | | 39 | | |
| 2 x 5 | Main | 7213.5 | 7213.5 | 1 | 66.32 | P < 0.001 ***** |
| | Error | 4132.9 | 108.8 | 38 | | |
| | Total | 11346.5 | | 39 | | |
| 3 x 4 | Main | 6.024 | 6.024 | 1 | 1.118 | 0.50 > P > 0.25 |
| | Error | 204.7 | 5.387 | 38 | | |
| | Total | 210.7 | | 39 | | |
| 3 x 5 | Main | 225.9 | 225.9 | 1 | 45.80 | P < 0.001 ***** |
| | Error | 187.4 | 4.932 | 38 | | |
| | Total | 413.3 | | 39 | | |
| 4 x 5 | Main | 158.2 | 158.2 | 1 | 29.78 | P < 0.001 ***** |
| | Error | 201.8 | 5.311 | 38 | | |
| | Total | 360.0 | | 39 | | |

(c) Definitive method for sedimentation

Based on the above experiments, the following was used as the definitive sedimentation procedure.

A levelled petri dish full of wet sediment (containing about 55 g wet sediment which is equivalent to about 43 g dry sediment) was added to 500 ml of synthetic sea water in a 500 ml stoppered glass measuring cylinder (internal diameter 4.75 cm). In all experiments the cylinder was inverted three times by hand before taking each sample. A sample size of 2 ml was removed at a depth of 10 cm from the centre of the cylinder at six different time intervals (5, 10, 20, 40, 60 and 120 seconds). Replicate samples were removed for each time. Allowance was made for the weight of salt in the solution.

(III) Testing how the removal of different amounts of organic material from sediment particles influences their sedimentation. The use of ashing and acid-cleaning

I have shown that ashing and acid-cleaning remove different amounts of organic material from each of the three sediments I used (p. 98). Under natural conditions, surfaces of sediment grains may have different amounts of organic material removed from them by such processes as rain, animal feeding, and microbial activity (see p. 183). The differential effects of ashing and acid-cleaning provide a simple experimental method for testing how the removal of different amounts of organic material influences sedimentation.

Three types of sediments were used in this series of experiments: Langbank, Ardmore and Rockware sediments.

The materials and methods in this section are divided into two parts. The first (1) describes the cleaning methods (i.e. ashing and acid-cleaning). The second (2) describes the staining and microscopic

record of the amount of materials on the surfaces of sediment particles before and after cleaning.

Sediments which had been ashed and acid-cleaned were then used in the sedimentation experiments.

1. Cleaning methods

The ashed and acid-cleaned sediments from the three above sediments were prepared as follows.

The three sediments were ashed and acid-cleaned separately by dry combustion at 600°C for 6 hours and by boiling in conc H_2SO_4 respectively. These two methods have been described previously in the organic carbon determination. See Materials and Methods, p. 51 in Section I, for details.

2. Staining and microscopic record of the amount of materials on sediment grain surfaces before and after cleaning

Samples from the three sediment types before cleaning (control) and after cleaning (ashing or acid-cleaning) were stained. The following sequence was used (Meadows and Anderson, 1968).

Samples were treated separately in 2% gluteraldehyde (prepared with sea water) for 15 minutes, transferred to 2% w/v of osmic acid for 2 minutes, and then placed in Bouin's solution for 2 minutes, and washed X2 with distilled water. The samples were then stained with carbol fuschin for 6 minutes. Finally, each sample was washed three times with distilled water and stored in diluted Bouin's solution until required.

(IV) Effects of different temperatures, salinities and wet and dry sediment on sedimentation

These experiments were conducted with Langbank sediment. The effects of three different salinities (0%, 50%, 100%), of three different temperatures (5°C, 10°C, 20°C), and of drying, were tested.

Wet and dry sediments were prepared by taking a large quantity of wet sieved sediment and dividing it into two equal parts. One part was maintained in aerated sea water to provide the wet sediment. The second was spread evenly on a tray and left at room temperature to dry, mixing it with a spatula three times a day. Water content was measured daily until no water was present. The results of the daily measurements of water content are shown in Figure 27. Dry and wet sediments were maintained at 5, 10 and 20°C as appropriate for 24 hours before the experiments. The water content of dry and wet sediments at the end of the 24 hours were measured in an initial experiment and are shown in Appendix II, Table 3, p. 460).

Forty-three gramme aliquots of dried sediment and the appropriate weight of the wet sediment (c. 55 g) were then used in the sedimentation experiments.

Different salinities (i.e. 50% and 100%) were prepared from synthetic sea water by dilution with distilled water as appropriate. The effects of temperature were obtained by running experiments in a cold room at 5°C, in an aquarium at 10°C, and at room temperature (20°C).

(V) Preparation of synthetic sea water

A known weight of natura sea salt (supplied by Philip Harris Biological Ltd, Oldmixon, Weston-Super-Mare, Avon) was dissolved in a known volume of tap water (local tap water is very soft and almost

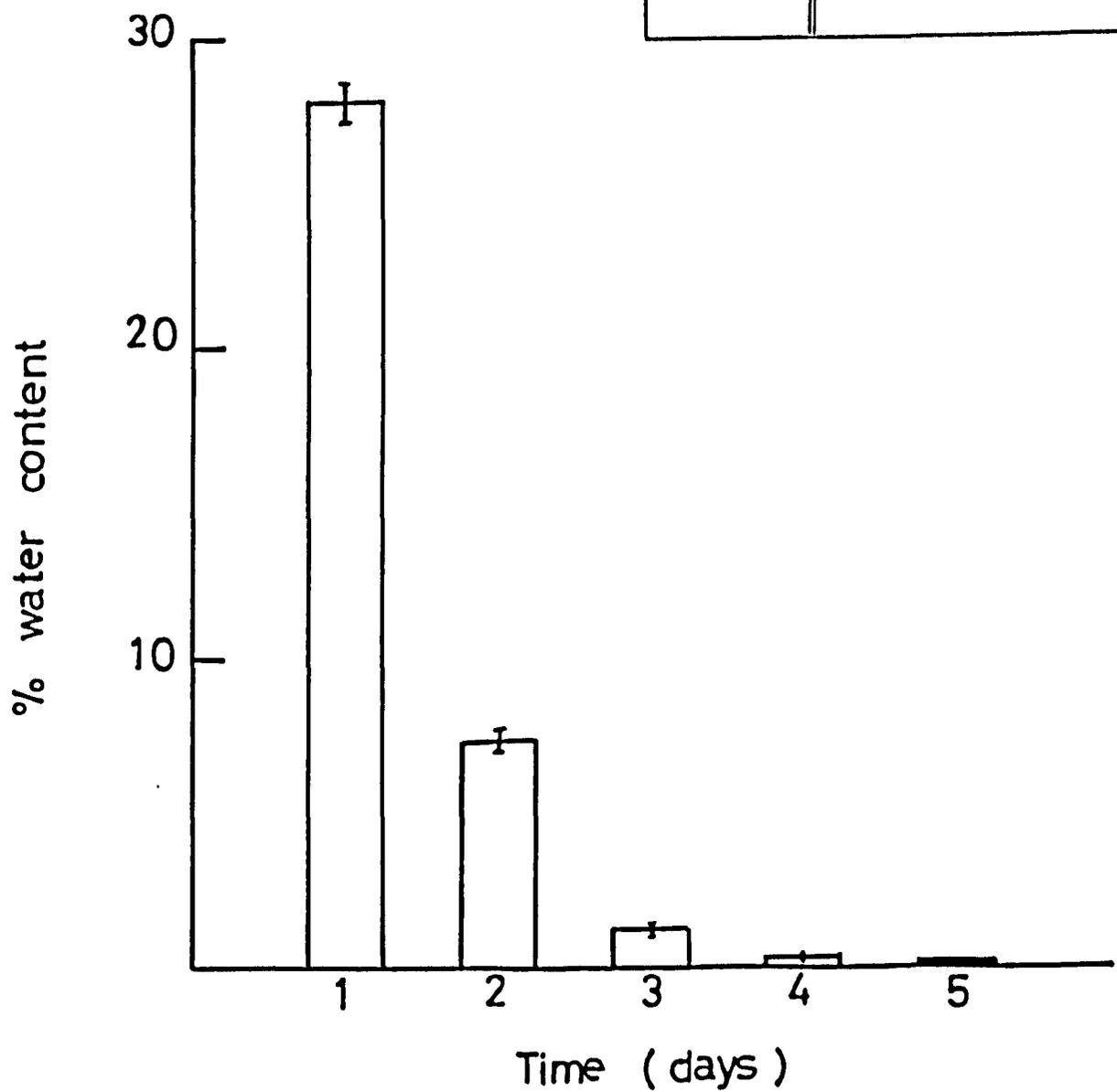
as pure as distilled water).

Salinity was measured by the silver nitrate technique. The titration was carried out in a 200 ml beaker against a white background in natural daylight. A magnetic stirrer was used. A 10 ml pipette sample of synthetic sea water and 15 ml of indicator-diluent solution (3.5 g of K_2CrO_4 in one litre of distilled water) were placed in the beaker. Titration of the solution was made from a 25 ml burette containing silver nitrate solution (0.28 N; prepared by dissolving 49.00 g of silver nitrate in one litre of distilled water). As the end-point approached, the localised red precipitate formed by the silver solution began to spread throughout the solution. At the end-point the pale greenish-yellow colour of the contents of the beaker changed to a full yellow and then became a definite pale red as the end-point was exceeded. The burette reading was recorded at the end-point. Salinity ($S^0/00$) was then found by comparing the burette reading with that in Table II (conversion of chlorosity to salinity) in Strickland and Parsons (1972, pp. 17, 284).

Figure 27

Experiment assessing the loss of water while air-drying
Langbank sediment. Means and standard deviations of
% water content loss during the drying period.

| Time (days) | (mean \pm s.d) |
|-------------|-----------------------|
| 1st | 27.77 ± 0.6285 |
| 2nd | 7.194 ± 0.4952 |
| 3rd | 1.134 ± 0.1439 |
| 4th | 0.3833 ± 0.007654 |
| 5th | 0.1550 ± 0.007697 |



SECTION II

Results

The Results in this section are divided into two parts. The first (I) tests how the removal of different amounts of organic material from sediment particles influences their sedimentation. The second (II) gives the effects of three different temperatures, three different salinities, and wet and dry sediment on sedimentation.

(I) The influence of the removal of different amounts of organic material from sediment particles on sedimentation

The Results in this part are divided into two divisions. The first (1) gives a microscopic record of the loss of organic material from sediment particles. The second (2) gives the effects of different amounts of organic material on sediment particles on sedimentation.

1. Microscopic record of the loss of organic material from sediment particles

The results of staining of Langbank, Ardmore and Rockware sediment particles before and after cleaning (i.e. ashing and acid-cleaning) are shown on Plates 6 to 23.

Plates 6, 7; 12, 13; 18 and 19 show the sediment particles from the control sediment (before treatments) of Langbank, Ardmore and Rockware sediments respectively. The stained material on the particles of Langbank and Ardmore sediments (Plates 6, 7; 12, 13) is much higher than that on Rockware particles (Plates 18, 19). This agrees with the organic carbon measurements (p. 98 ; Table 13; row 1).

Ashing and acid-cleaning reduce the stained material on the three sediments (Plates 8-11, 14-17, 20-23). This effect is only clearly

noticeable on the micrographs of the Langbank and Ardmore sediments. The difference - although present - is not obvious on the photomicrographs of the Rockware sediment. The effects of ashing and acid-cleaning agrees with the organic carbon measurements (p. 98 ; Table 13, rows 2, 3).

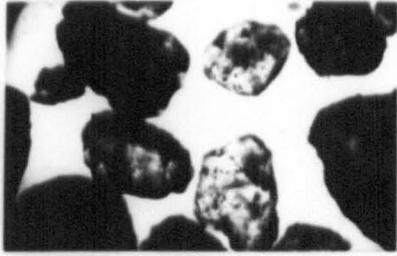
Plates 6-11

Langbank sediment. Stained.

Top row: Plates 6, 7, control.

Middle row: Plates 8, 9, ashed.

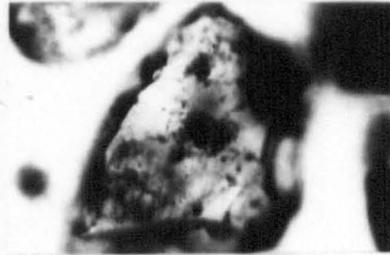
Bottom row: Plates 10, 11, acid-cleaned.



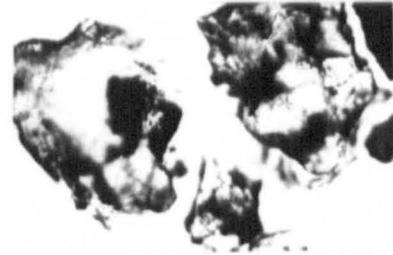
6



7



8



9



10



11

Plates 12-17

Ardmore sediment. Stained.

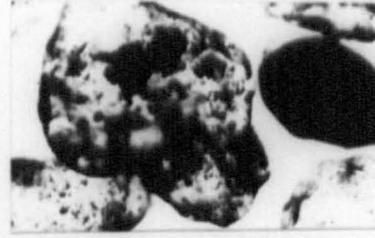
Top row: Plates 12, 13, control.

Middle row: Plates 14, 15, ashed.

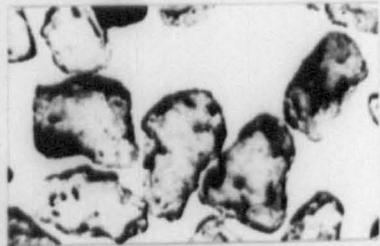
Bottom row: Plates 16, 17, acid-cleaned.



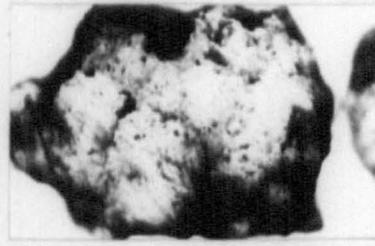
12



13



14



15



16



17

212.

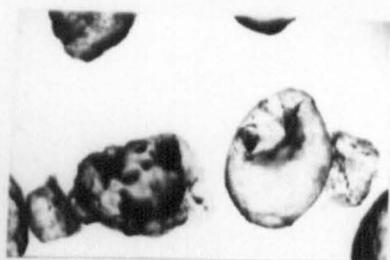
Plates 18-23

Rockware sediment. Stained.

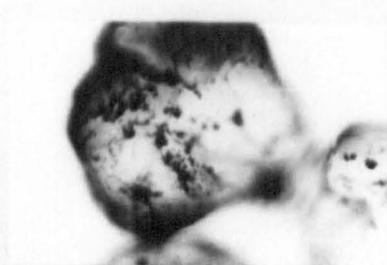
Top row: Plates 18, 19, control.

Middle row: Plates 20, 21, ashed.

Bottom row: Plates 22, 23, acid-cleaned.



18



19



20



21



22



23

2. Effects of different amounts of organic material on sediment particles on sedimentation

The results in this part are divided into two divisions. The first (a) gives a graphical presentation of results. The second (b) gives the statistical analyses.

(a) Graphical presentation of results

The results of this experimental series are presented in Table 28 and Figures 28, 29 and 30.

Table 28 shows the means and standard deviations of suspended weights of the three types of sediments (Langbank, Ardmore and Rockware). It also shows the different treatments (ashed, acid-cleaned, and control), and the different time intervals. The data from Table 28 are plotted in Figures 28, 29 and 30.

The removal of organic material by ashing and acid-cleaning dramatically increases sedimentation. The effect is most noticeable in the Langbank and Ardmore sediments (Figures 28 and 29). Ashed sediments sedimented most quickly, followed by acid-cleaned sediments, followed by the controls. These differences match the organic contents (ashed < acid-cleaned < control). Similar but much less obvious effects were noted with Rockware sediment.

TABLE 28. Means and standard deviations of suspended weights (mg/ml) of Langbank (LBM), Ardmore (ARD) and Rockware (RWS) sediments. With two cleaning methods (ashed/acid-cleaned) and the control (uncleaned) sediments at different time intervals. (Each value represents the means of ten replicates.)

| Sedi- ment type | Treatment | Time intervals (seconds) | | | | | |
|-----------------------|------------------|--------------------------|----------------------|-----------------------|------------------------|--------------------------|--------------------------|
| | | 5 | 10 | 20 | 40 | 60 | 120 |
| LBM | Control | 14.71 + 4.342 | 9.550 + 3.062 | 7.970 + 1.940 | 6.950 + 2.519 | 6.270 + 1.837 | 2.660 + 1.645 |
| | Ashed | 2.790 + 0.7660 | 1.630 + 1.150 | 0.6600 + 0.2989 | 0.5100 + 0.3479 | 0.4500 + 0.4138 | 0.04000 + 0.04216 |
| | Acid- cleaned | 5.540 + 1.657 | 4.670 + 2.189 | 4.140 + 1.385 | 3.718 + 3.092 | 1.160 + 1.150 | 0.2360 + 0.4048 |
| ARD | Control | 15.39 + 3.561 | 9.162 + 2.745 | 7.250 + 0.9043 | 6.210 + 1.782 | 5.410 + 1.352 | 2.448 + 1.379 |
| | Ashed | 2.855 + 0.5199 | 1.445 + 0.5252 | 0.7000 + 0.2539 | 0.5200 + 0.09189 | 0.3400 + 0.06583 | 0.05500 + 0.04478 |
| | Acid- cleaned | 5.435 + 1.067 | 3.993 + 1.357 | 3.955 + 0.05986 | 3.230 + 0.3155 | 1.015 + 0.1270 | 0.03700 + 0.03683 |
| RWS | Control | 6.436 + 5.034 | 2.540 + 1.944 | 1.480 + 1.255 | 0.8020 + 0.3296 | 0.03620 + 0.03389 | 0.02620 + 0.02601 |
| | Ashed | 4.980 + 4.640 | 1.460 + 0.5232 | 0.8300 + 0.8006 | 0.6200 + 0.4295 | 0.03100 + 0.01542 | 0.01200 + 0.08165 |
| | Acid- cleaned | 2.800 + 2.364 | 1.430 + 0.8962 | 0.5590 + 0.3734 | 0.4180 + 0.4138 | 0.03600 + 0.004452 | 0.01500 + 0.005270 |

Figure 28

Means and standard deviations of suspended weights (mg dry weight/ml) of Langbank sediment of uncleaned (control) and cleaned (ashed and acid-cleaned) sediment at different time intervals (5, 10, 20, 40, 60 and 120 seconds).

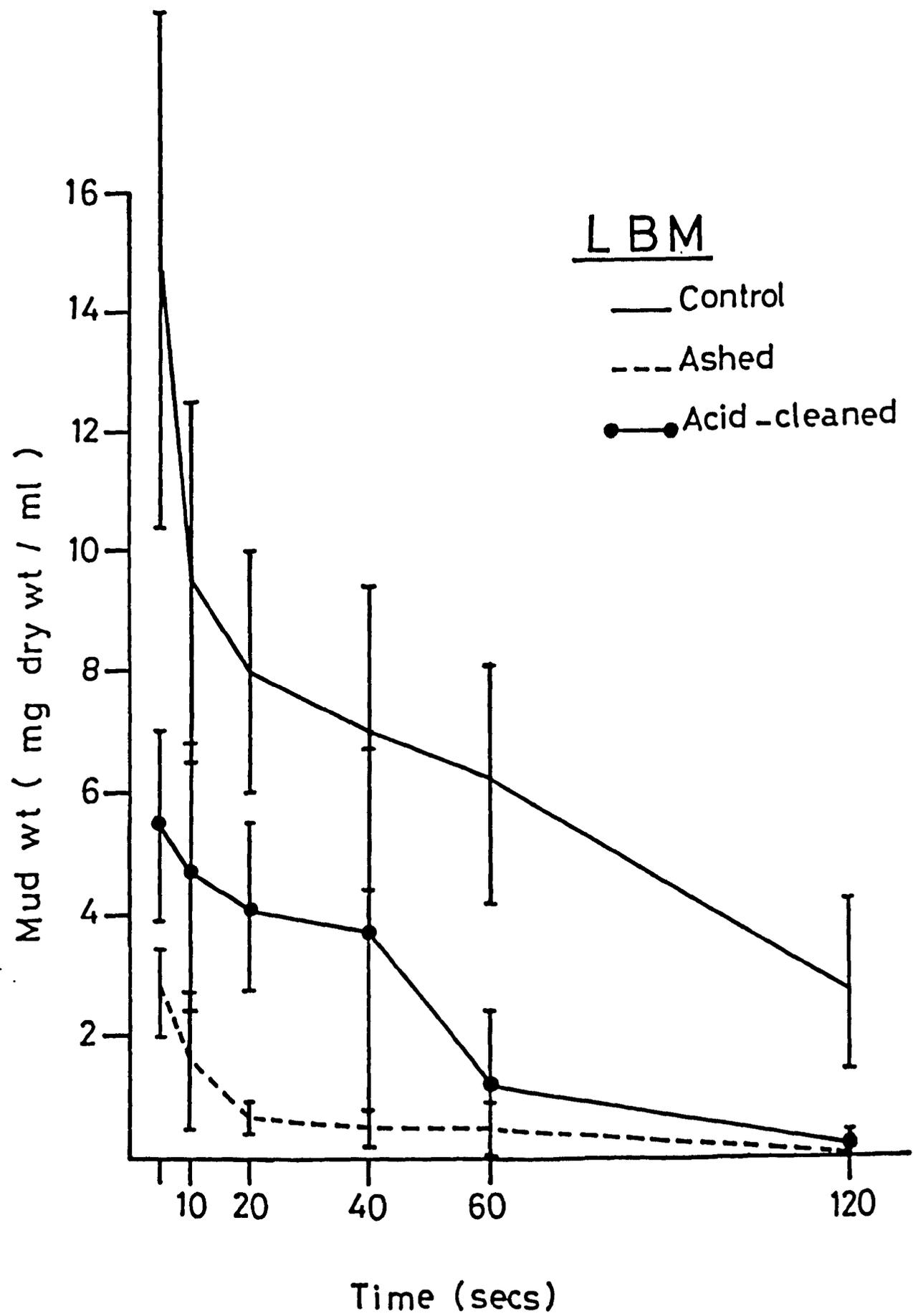


Figure 29

Means and standard deviations of suspended weights of Ardmore sediment of uncleaned (control) and cleaned (ashed and acid-cleaned) sediment at different time intervals (5, 10, 20, 40, 60 and 120 seconds).

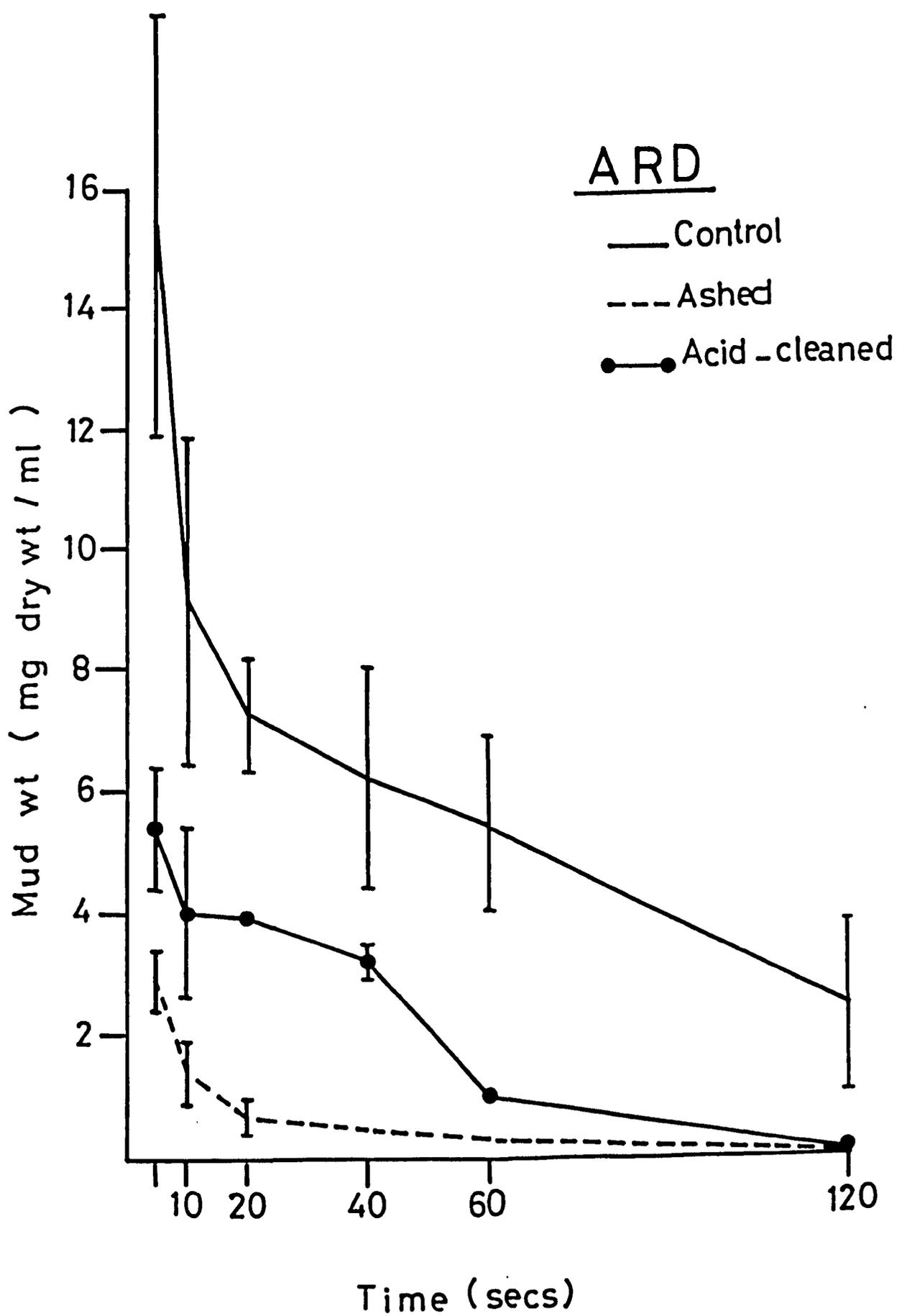
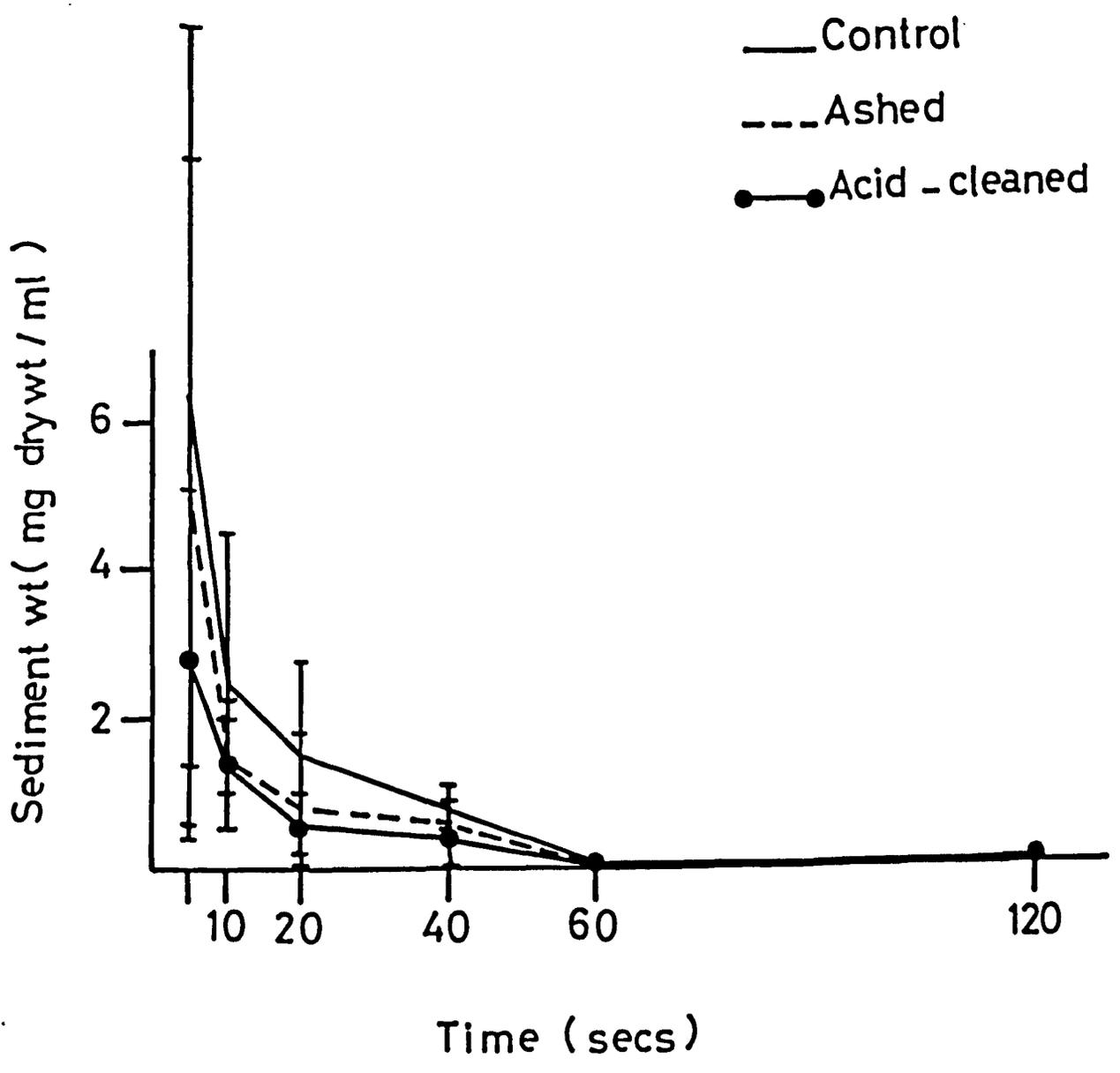


Figure 30

Means and standard deviations of suspended weights of Rockware sediment of uncleaned (control) and cleaned (ashed and acid-cleaned) sediments at different time intervals (5, 10, 20, 40, 60 and 120 seconds).

RWS



(b) Statistical analysis of results

The variation of suspended weight of each sediment with different treatments (control/ashed/acid-cleaned) and sampling times was analysed by three two-way analyses of variance - one for each of the three sediments. In each two-way analysis of variance, Factor A was the three different treatments (control, ashed, acid-cleaned) and Factor B was the six different time intervals (5, 10, 20, 40, 60 and 120 seconds).

The results of these analyses are shown in Appendix II, Tables 4, 5, 6, pp. 461, 462, 463. Highly significant interactions were found with both Langbank and Ardmore sediments (both $P < 0.001$), and a significant interaction was found in Rockware sediment ($0.05 > P > 0.025$).

A series of breakdown one-way analyses were therefore carried out on the data for each sediment type and each treatment, a one-way anovar was applied to the weights at the different time intervals. This resulted in nine one-way anovars, three for each sediment type. Tables 29, 30 and 31 show the results of these analyses. As is to be expected, highly significant differences were found with all the sediments and treatments between the different times ($P < 0.001$). This reflects the decrease in weight of sediment in suspension with time.

Another series of breakdown one-way analyses were carried out for each sediment type and each time, to compare the suspended weights found with different treatments. This resulted in eighteen one-way anovars, six for each of the three sediment types (the six representing the six times). Tables 32, 33 and 34 show the results of these analyses for Langbank, Ardmore and Rockware sediments respectively. With Langbank sediment (Table 32) and Ardmore sediment

(Table 33), highly significant differences in suspended weight were found between different treatments ($P < 0.001$). With Rockware sediment (Table 34), no significant differences in suspended weight were found at 5 and 10 seconds ($0.25 > P > 0.10$), and no definite differences in suspended weight were found at 20 and 40 seconds ($0.10 > P > 0.05$). Highly significant differences in suspended weight were found at 60 and 120 seconds ($P < 0.001$). These statistical analyses show that the removal of organic material by ashing and acid-cleaning has a dramatic effect on sedimentation of Langbank and Ardmore sediments, but a much smaller effect on Rockware sediments.

TABLE 29. One-way anovars testing the variation in suspended weights of Langbank sediment with time (5, 10, 20, 40, 60 and 120 seconds) during sedimentation for the control, ashed, and acid-cleaned treatments.

| Experimental treatment | Factors | | Sum of squares Ss | Means squares Ms | D.F | F-ratio | Probability |
|------------------------|-----------|-----------|----------------------|---------------------|-----|---------|-------------|
| | Time secs | | | | | | |
| Control | 5 secs | Main | 0.0008004 | 0.0001601 | 5 | 21.62 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.0003998 | 0.000007403 | 54 | | ***** |
| | 60 secs | | | | | | |
| | 120 secs | | | | | | |
| Ashed | 5 secs | Main | 0.00005180 | 0.00001036 | 5 | 27.10 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.00002064 | 0.0000003822 | 54 | | ***** |
| | 60 secs | | | | | | |
| | 120 secs | | | | | | |
| Acid-cleaned | 5 secs | Main | 0.0002148 | 0.00004296 | 5 | 13.86 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.0001674 | 0.000003100 | 54 | | ***** |
| | 60 secs | | | | | | |
| | 120 secs | | | | | | |
| | Total | 0.0003822 | | | 59 | | |

225.

TABLE 30. One-way anovars testing the variation in suspended weights of Ardmore sediment with time (5, 10, 20, 40, 60 and 120 seconds) during sedimentation for the control, ashed, and acid-cleaned treatments.

| Experimental treatment | Factors | | Sum of squares Ss | Means squares Ms | D.F | F-ratio | Probability |
|------------------------|-----------|-------|-------------------|------------------|-----|---------|-------------|
| | Time secs | | | | | | |
| Control | 5 secs | Main | 0.0009651 | 0.0001930 | 5 | 41.45 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.0002514 | 0.000004656 | 54 | | |
| | 60 secs | Total | 0.001216 | | 59 | | ***** |
| | 120 secs | | | | | | |
| Ashed | 5 secs | Main | 0.00005287 | 0.00001057 | 5 | 101.5 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.000005628 | 0.0000001042 | 54 | | |
| | 60 secs | Total | 0.00005850 | | 59 | | ***** |
| | 120 secs | | | | | | |
| Acid-cleaned | 5 secs | Main | 0.0002058 | 0.00004116 | 5 | 78.99 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.00002814 | 0.0000005211 | 54 | | |
| | 60 secs | Total | 0.0002340 | | 59 | | ***** |
| | 120 secs | | | | | | |

TABLE 31. One-way anovars testing the variation in suspended weights of Rockware sediment with time (5, 10, 20, 40, 60 and 120 seconds) during sedimentation for control, ashed, and acid-cleaned treatments.

| Experimental treatment | Factors | | Sum of squares Ss | Means squares Ms | D.F | F-ratio | Probability |
|------------------------|-----------|-------|-------------------|------------------|-----|---------|-------------|
| | Time secs | | | | | | |
| Control | 5 secs | Main | 0.0003108 | 0.00006216 | 5 | 12.11 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.0002772 | 0.000005134 | 54 | | |
| | 60 secs | Total | 0.0005880 | | 59 | | ***** |
| | 120 secs | | | | | | |
| Ashed | 5 secs | Main | 0.0001601 | 0.00003201 | 5 | 8.438 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.0002049 | 0.000003794 | 54 | | |
| | 60 secs | Total | 0.0003650 | | 59 | | ***** |
| | 120 secs | | | | | | |
| Acid-cleaned | 5 secs | Main | 0.00005784 | 0.00001157 | 5 | 10.33 | P < 0.001 |
| | 10 secs | | | | | | |
| | 20 secs | | | | | | |
| | 40 secs | Error | 0.00006046 | 0.000001120 | 54 | | |
| | 60 secs | Total | 0.0001183 | | 59 | | ***** |
| | 120 secs | | | | | | |

TABLE 32. One-way anovars testing the variation in suspended weights of Langbank sediment with different treatments (control, ashed, and acid-cleaned) during sedimentation at the six different time intervals (5, 10, 20, 40, 60 and 120 seconds).

| Time | Factors | | Sum of squares S _s | Means squares M _s | D.F | F-ratio | Probability |
|-------------|--------------|-------|----------------------------------|---------------------------------|-----|---------|------------------------|
| 5 secs | Control | Main | 0.0009788 | 0.0003896 | 2 | 52.67 | P < 0.001 ***** |
| | Ashed | Error | 0.0007791 | 0.000007397 | 27 | | |
| | Acid-cleaned | Total | 0.0017579 | | 29 | | |
| 10 secs | Control | Main | 0.0003193 | 0.0001596 | 2 | 30.91 | P < 0.001 ***** |
| | Ashed | Error | 0.0001394 | 0.000005164 | 27 | | |
| | Acid-cleaned | Total | 0.0004587 | | 29 | | |
| 20 secs | Control | Main | 0.0002674 | 0.0001337 | 2 | 69.52 | P < 0.001 ***** |
| | Ashed | Error | 0.00005193 | 0.000001923 | 27 | | |
| | Acid-cleaned | Total | 0.0003193 | | 29 | | |
| 40 secs | Control | Main | 0.0002111 | 0.0001056 | 2 | 22.42 | P < 0.001 ***** |
| | Ashed | Error | 0.0001271 | 0.000004708 | 27 | | |
| | Acid-cleaned | Total | 0.0003382 | | 29 | | |
| 60 secs | Control | Main | 0.0002016 | 0.0001008 | 2 | 62.10 | P < 0.001 ***** |
| | Ashed | Error | 0.00004383 | 0.000001623 | 27 | | |
| | Acid-cleaned | Total | 0.0002455 | | 29 | | |
| 120 secs | Control | Main | 0.00004253 | 0.00002126 | 2 | 22.26 | P < 0.001 ***** |
| | Ashed | Error | 0.00002580 | 0.0000009554 | 27 | | |
| | Acid-cleaned | Total | 0.00006833 | | 29 | | |

TABLE 33. One-way anovars testing the variations in suspended weights of Ardmore sediment with different treatments (control, ashed, and acid-cleaned) during sedimentation at the six different time intervals (5, 10, 20, 40, 60 and 120 seconds).

| Time | Factors | | Sum of squares S _s | Means squares M _s | D.F | F-ratio | Probability |
|-------------|--------------|-------|----------------------------------|---------------------------------|-----|---------|------------------------|
| 5 secs | Control | Main | 0.0008763 | 0.0004381 | 2 | 93.29 | P < 0.001 ***** |
| | Ashed | Error | 0.0001268 | 0.000004697 | 27 | | |
| | Acid-cleaned | Total | 0.001003 | | 29 | | |
| 10 secs | Control | Main | 0.0003963 | 0.0001546 | 2 | 47.93 | P < 0.001 ***** |
| | Ashed | Error | 0.00008709 | 0.000003226 | 7 | | |
| | Acid-cleaned | Total | 0.0003963 | | 29 | | |
| 20 secs | Control | Main | 0.0002145 | 0.0001073 | 2 | 363.3 | P < 0.001 ***** |
| | Ashed | Error | 0.000007972 | 0.0000002953 | 27 | | |
| | Acid-cleaned | Total | 0.0002225 | | 29 | | |
| 40 secs | Control | Main | 0.0001620 | 0.00008100 | 2 | 74.04 | P < 0.001 ***** |
| | Ashed | Error | 0.00002954 | 0.000001094 | 27 | | |
| | Acid-cleaned | Total | 0.0001915 | | 29 | | |
| 60 secs | Control | Main | 0.0001516 | 0.00007579 | 2 | 123.1 | P < 0.001 ***** |
| | Ashed | Error | 0.00001663 | 0.0000006159 | 27 | | |
| | Acid-cleaned | Total | 0.0001682 | | 29 | | |
| 120 secs | Control | Main | 0.00003847 | 0.00001923 | 2 | 30.27 | P < 0.001 ***** |
| | Ashed | Error | 0.00001715 | 0.0000006353 | 27 | | |
| | Acid-cleaned | Total | 0.00005562 | | 29 | | |

TABLE 34. One-way anovars testing the variation in suspended weights of Rockware sediment with different treatments (control, ashed, and acid-cleaned) during sedimentation at the six different time intervals (5, 10, 20, 40, 60 and 120 seconds).

| Time | Factors | | Sum of squares Ss | Means squares Ms | D.F | F-ratio | Probability |
|-------------|--------------|-------|----------------------|---------------------|-----|---------|------------------------|
| 5 secs | Control | Main | 0.00006698 | 0.00003349 | 2 | 1.915 | 0.25 > P > 0.10 |
| | Ashed | Error | 0.0004722 | 0.00001749 | 27 | | |
| | Acid-cleaned | Total | 0.0005391 | | 29 | | |
| 10 secs | Control | Main | 0.000007998 | 0.000003999 | 2 | 2.470 | 0.25 > P > 0.10 |
| | Ashed | Error | 0.00004371 | 0.000001619 | 27 | | |
| | Acid-cleaned | Total | 0.00005171 | | 29 | | |
| 20 secs | Control | Main | 0.000004489 | 0.000002244 | 2 | 2.858 | 0.10 > P > 0.05 |
| | Ashed | Error | 0.00002120 | 0.0000007851 | 27 | | |
| | Acid-cleaned | Total | 0.00002569 | | 29 | | |
| 40 secs | Control | Main | 0.0000008633 | 0.0000004317 | 2 | 2.640 | 0.10 > P > 0.05 |
| | Ashed | Error | 0.000004414 | 0.0000001635 | 27 | | |
| | Acid-cleaned | Total | 0.000005278 | | 29 | | |
| 60 secs | Control | Main | 0.000002258 | 0.000001129 | 2 | 26.28 | P < 0.001 ***** |
| | Ashed | Error | 0.000001160 | 0.00000004297 | 27 | | |
| | Acid-cleaned | Total | 0.000003419 | | 29 | | |
| 120 secs | Control | Main | 0.00000008067 | 0.00000004033 | 2 | 17.56 | P < 0.001 ***** |
| | Ashed | Error | 0.00000006200 | 0.00000002296 | 27 | | |
| | Acid-cleaned | Total | 0.0000001427 | | 29 | | |

The previous statistical analyses do not answer the question: "What differences are there between the sedimentation of the three sediment types?" The following analyses are directed towards answering this question. Three two-way analyses of variance were applied comparing the three sediment types.

In all the three analyses, Factor A was the three sediment types (Langbank, Ardmore and Rockware sediments), and Factor B was the six time intervals. The first two-way analysis compared the control sediments; the second, the ashed sediments and the third, the acid-cleaned sediments. The first analysis (Appendix II, Table 7, p. 464) shows that there was a significant interaction between the three control sediments and the six time intervals ($0.025 > P > 0.01$). Therefore, a series of six breakdown one-way analyses were carried out for each of the six times (Table 35). Highly significant differences in suspended weight were found between the three control sediments at all time intervals ($P < 0.001$).

The second two-way analysis (Appendix II, Table 8, p. 465) shows the interaction factor was possibly significant. Therefore a series of six breakdown one-way analyses were carried out for each of the six times. Table 36 shows this. No significant differences in suspended weight were found at all time intervals ($P > 0.10$) except at 120 seconds ($0.05 > P > 0.025$).

As in the two previous two-way analyses of variance, the third two-way analysis showed that there was a significant interaction ($P < 0.001$) (Appendix II, Table 9, p. 466). Therefore, a series of six breakdown one-way analyses were carried out for each of the six times (Table 37). Significant differences in suspended weight were found between the three acid-cleaned sediments at all time intervals ($P < 0.005$), except at 120 seconds ($0.10 > P > 0.05$).

From these two-way and one-way analyses, the following points can be concluded. The significant differences in suspended weight show that the three control sediments behaved totally differently during sedimentation. This is also true with the acid-cleaned sediments. However, there were no significant differences in the sedimentation between the three ashed sediments.

These results are surprising, since it had been expected that the acid-cleaned and ashed sediments would behave similarly.

Two alternatives had been expected: (i) some differences between Langbank, Ardmore, and Rockware sediments would have remained after acid-cleaning and ashing, or (ii) no differences would have remained. In fact, alternative (i) was true for acid-cleaned, while alternative (ii) was true for ashed sediment. It is not immediately obvious why the acid-cleaned and ashed sediments should have behaved differently. However, a careful consideration of the facts suggested a probable explanation which is presented in the Discussion on page 137 .

TABLE 35. One-way anovars of the three control sediments (Langbank, Ardmore and Rockware sediments) at different time intervals (5, 10, 20, 40, 60 and 120 seconds).

| Time | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|------|---------|----------------|--------------|-----|---------|--------------------|
| 5 | Main | 452.6 | 226.3 | 2 | 9.144 | P < 0.001 ***** |
| | Error | 668.2 | 24.75 | 27 | | |
| | Total | 1120.9 | | 29 | | |
| 10 | Main | 310.5 | 155.2 | 2 | 22.51 | P < 0.001 ***** |
| | Error | 186.2 | 6.896 | 27 | | |
| | Total | 496.7 | | 29 | | |
| 20 | Main | 253.1 | 126.6 | 2 | 61.69 | P < 0.001 ***** |
| | Error | 55.39 | 2.051 | 27 | | |
| | Total | 308.5 | | 29 | | |
| 40 | Main | 274.5 | 137.3 | 2 | 42.76 | P < 0.001 ***** |
| | Error | 86.67 | 3.210 | 27 | | |
| | Total | 361.2 | | 29 | | |
| 60 | Main | 229.03 | 114.5 | 2 | 66.01 | P < 0.001 ***** |
| | Error | 46.84 | 1.735 | 27 | | |
| | Total | 275.9 | | 29 | | |
| 120 | Main | 42.66 | 21.33 | 2 | 13.89 | P < 0.001 ***** |
| | Error | 41.47 | 1.536 | 27 | | |
| | Total | 84.13 | | 29 | | |

TABLE 36. One-way anovars of the three ashed sediments (Langbank, Ardmore and Rockware sediments) at different time intervals (5, 10, 20, 40, 60 and 120 seconds).

| Time | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|------|---------|----------------|--------------|-----|---------|------------------|
| 5 | Main | 31.05 | 15.53 | 2 | 2.080 | 0.25 > P > 0.10 |
| | Error | 201.5 | 7.464 | 27 | | |
| | Total | 232.6 | | 29 | | |
| 10 | Main | 0.2112 | 0.1056 | 2 | 0.1691 | P > 0.75 |
| | Error | 16.86 | 0.6243 | 27 | | |
| | Total | 17.07 | | 29 | | |
| 20 | Main | 0.1580 | 0.0790 | 2 | 0.2982 | 0.75 > P > 0.50 |
| | Error | 7.153 | 0.2649 | 27 | | |
| | Total | 7.311 | | 29 | | |
| 40 | Main | 0.07400 | 0.03700 | 2 | 0.3536 | 0.75 > P > 0.50 |
| | Error | 2.825 | 0.1046 | 27 | | |
| | Total | 2.899 | | 29 | | |
| 60 | Main | 0.3687 | 0.1843 | 2 | 1.837 | 0.25 > P > 0.10 |
| | Error | 2.709 | 0.1003 | 27 | | |
| | Total | 3.078 | | 29 | | |
| 120 | Main | 0.03617 | 0.0181 | 2 | 5.191 | 0.05 > P > 0.025 |
| | Error | 0.09405 | 0.003483 | 27 | | |
| | Total | 0.1302 | | 29 | | |

*

TABLE 37. One-way anovars of the acid-cleaned sediments (Langbank, Ardmore and Rockware sediments) at different time intervals (5, 10, 20, 40, 60 and 120 seconds).

| Time | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|------|---------|----------------|--------------|-----|---------|---------------------------|
| 5 | Main | 48.21 | 24.10 | 2 | 7.632 | 0.005 > P > 0.001 **** |
| | Error | 85.27 | 3.158 | 27 | | |
| | Total | 133.5 | | 29 | | |
| 10 | Main | 58.42 | 29.21 | 2 | 11.74 | P < 0.001 ***** |
| | Error | 67.19 | 2.488 | 27 | | |
| | Total | 125.6 | | 29 | | |
| 20 | Main | 81.30 | 40.65 | 2 | 59.19 | P < 0.001 ***** |
| | Error | 18.54 | 0.6868 | 27 | | |
| | Total | 99.85 | | 29 | | |
| 40 | Main | 63.45 | 31.73 | 2 | 9.671 | P < 0.001 ***** |
| | Error | 88.57 | 3.280 | 27 | | |
| | Total | 152.0 | | 29 | | |
| 60 | Main | 7.476 | 3.738 | 2 | 8.364 | 0.005 > P > 0.001 **** |
| | Error | 12.07 | 0.4469 | 27 | | |
| | Total | 19.54 | | 29 | | |
| 120 | Main | 0.2964 | 0.1482 | 2 | 2.690 | 0.10 > P > 0.05 |
| | Error | 1.488 | 0.0551 | 27 | | |
| | Total | 1.784 | | 29 | | |

(II) Effects of three different temperatures, three different salinities and wet and dry sediment on sedimentation

The results of this part are divided into four sections. The first gives the graphical presentation of results, the second, the justification for statistical methodology, the third, the analyses of variance, and the fourth, a linear regression analysis.

1. Graphical presentation of results

The results of this part are presented in Figures 31, 32 and 33. The original data are given in Appendix II, Table 10, p.467 .

In all cases dry sediment settled much more quickly than wet sediment. Sedimentation occurred quickly at the lowest salinity tested (0%), but at higher salinities (50% and 100%), sedimentation was slower (Figures 31, 32 and 33).

Temperature had a slight effect on sedimentation. At 20°C, sedimentation occurred slightly quicker than at 5°C or at 10°C, while at 10°C, sedimentation was slower than at 20°C or 5°C.

2. Justification for statistical methodology

Data were analysed by two statistical methods. These were analysis of variance (both one-way and two-way), and regression analyses. Both methods have their advantages and disadvantages as follows.

Analyses of variance allow one to compare differences between treatments even though there is no linear trend, and also allows one to compare between pairs of treatments in turn when there are more than two treatments.

Figure 31

Langbank sediment. Relationship between suspended sediment (mg/ml) and time. Points represent means and standard deviations of five replicate readings. Some standard deviations were so small that they could not be seen.

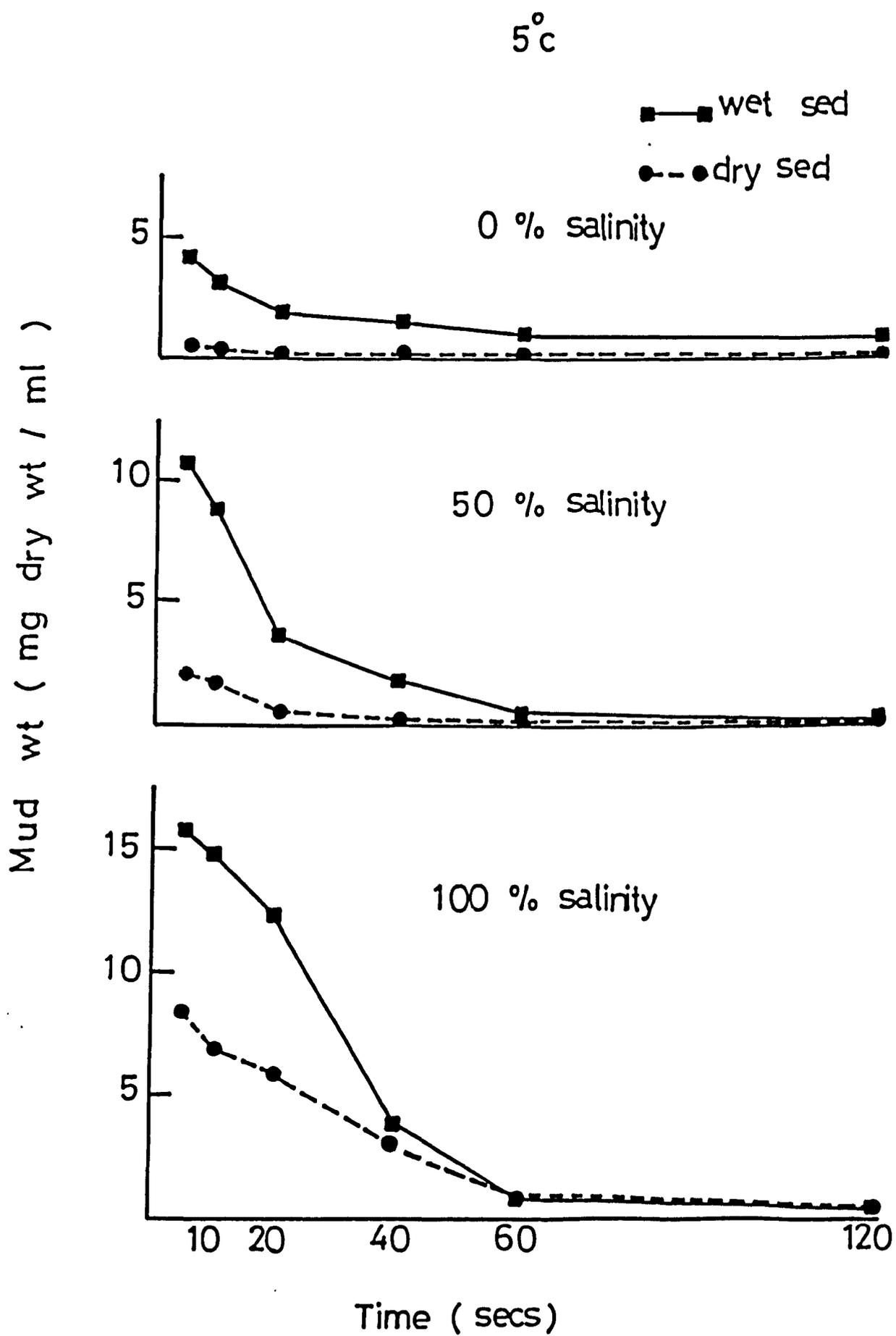


Figure 32

Langbank sediment. Relationship between suspended sediment (mg/ml) and time. Points represent means and standard deviations of five replicate readings. Some standard deviations were so small that they could not be seen.

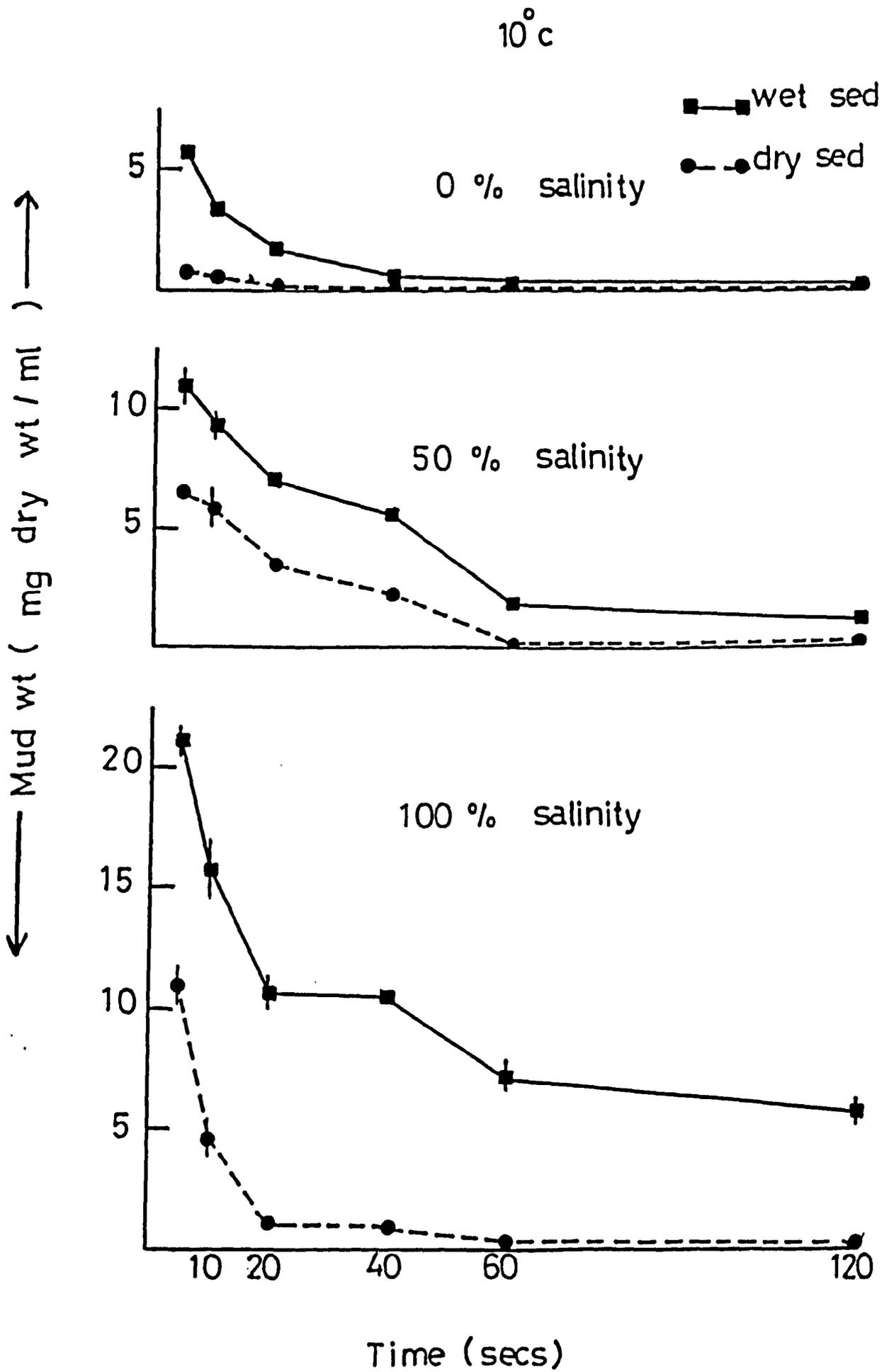
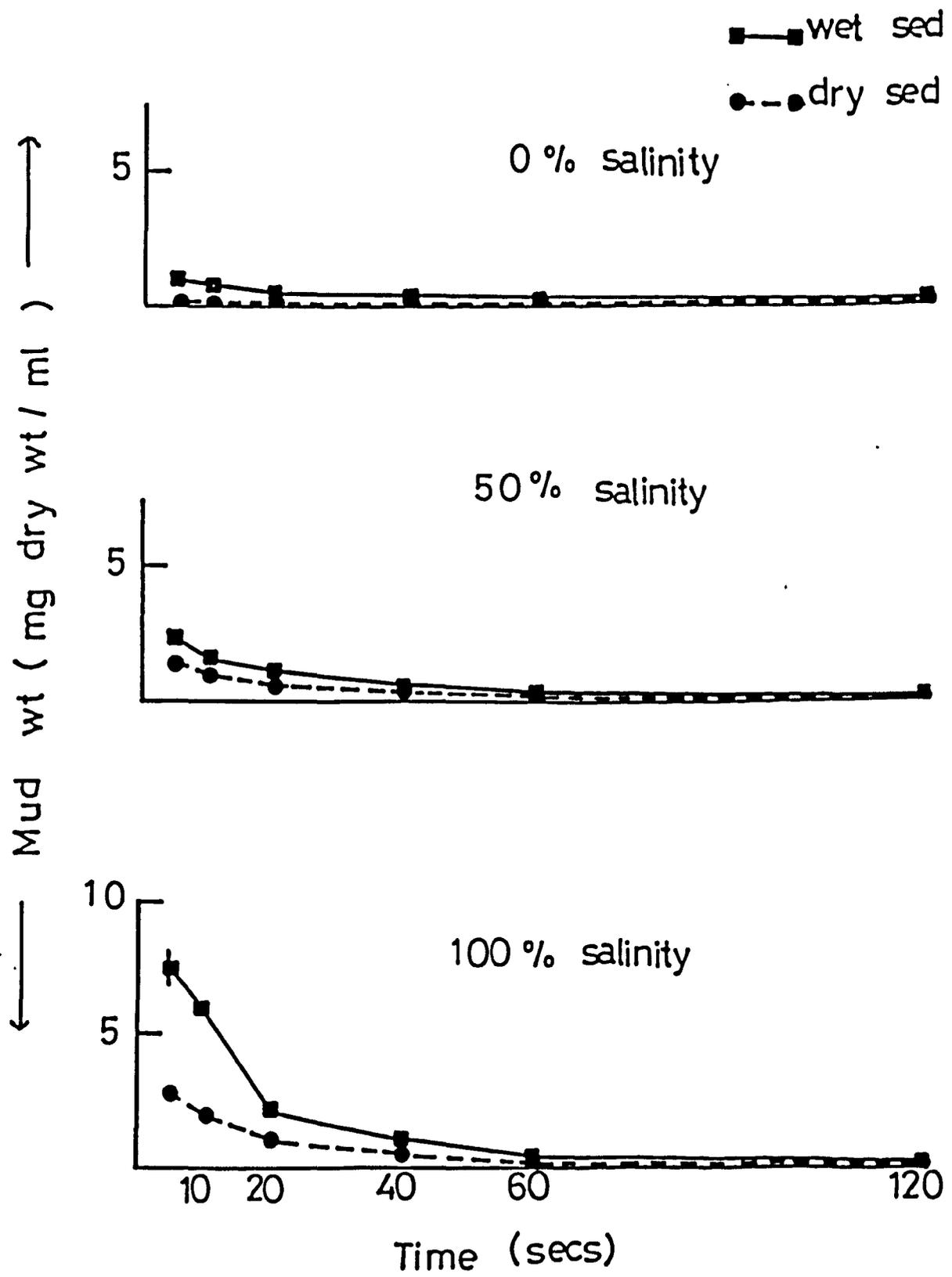


Figure 33

Langbank sediment. Relationship between suspended sediment (mg/ml) and time. Points represent means and standard deviations of five replicate readings. Some standard deviations were so small that they could not be seen.

20° c



On the other hand, if the data shows a linear trend, or can be transformed to a linear trend, then regression analysis can be applied. Here an overall relationship of the form $y = mx + c$ allows one to accurately predict y values for any given x , and also to consider the intercept of the line on the y axis and on the x axis.

Because of the above advantages and disadvantages of each method, I analysed the data by both methods.

3. Application of analysis of variance to results

Variations of suspended weight with wet/dry sediments, at different temperatures, salinities and at different time intervals were analysed by three series of two-way anovars. The first series of two-way analyses compared the effect of wet and dry sediments against time, the second, the three different salinities against time and the third, the three different temperatures against time. These three series are presented below.

(a) First series of anovars - wet/dry effect

In the first series of two-way analyses of variance, Factor A was wet/dry sediments and Factor B the six time intervals. These analyses were carried out for each temperature and salinity. Results of these nine analyses are shown in Appendix II, Tables 11-19, pp. 468-476. Significant interactions were found in eight out of the nine analyses. The interaction was not significant with 10°C and 50% salinities ($0.25 > P > 0.10$) (Appendix II, Table 15, p. 472).

Because of the significant interactions, a series of breakdown one-way analyses were carried out comparing wet and dry sediments at the three different temperatures, three different salinities and at the six time intervals. This resulted in fifty-four one-way anovars (Appendix II, Tables 20-28, pp. 477 - 485). The F-ratios from

these one-way anovars are shown in Table 38.

As expected from Figures 31-33, highly significant differences in suspended weight were found in forty-four out of the fifty-four comparisons ($P < 0.005$), significant differences were found in five anovars ($0.05 > P > 0.01$). No significant differences were found in four anovars and no definite result was found in one case only. These statistical analyses show that in general, wet sediments sediment more slowly than dry ones.

TABLE 38. Statistical comparisons of wet and dry suspended sediment (mg/ml). F-ratios from fifty-four 1 x 2 one-way anovars (Appendix II, Tables 20-28, pp. 477-485).

| Salinity | 5°C | | | | | | 10°C | | | | | |
|----------|-----------------|----------------|----------------|-----------------|----------------|----------------|----------------|---------------|----------------|----------------|----------------|----------------|
| | Time (secs) | | | | | | Time (secs) | | | | | |
| | 5 | 10 | 20 | 40 | 60 | 120 | 5 | 10 | 20 | 40 | 60 | 120 |
| 0% | 3394.0 ***** | 462.7 ***** | 271.1 ***** | 5660.5 ***** | 332.3 ***** | 953.0 ***** | 113.9 ***** | 6.346 * | 91.06 ***** | 24.87 **** | 68.30 ***** | 188.9 ***** |
| 50% | 54.60 ***** | 216.7 ***** | 149.1 ***** | 286.5 ***** | 325.0 ***** | 53.37 ***** | 5.316 | 3.041 | 30.85 ***** | 7.771 ** | 318.3 ***** | 39.04 ***** |
| 100% | 26.14 ***** | 964.9 ***** | 59.33 ***** | 1.081 | 1.154 | 2.951 | 32.98 ***** | 24.98 **** | 43.98 ***** | 945.2 ***** | 27.70 ***** | 19.66 ***** |

***** = $P < 0.001$; **** = $0.005 > P > 0.001$; ** = $0.025 > P > 0.01$; * = $0.05 > P > 0.025$

(Continued overleaf)

TABLE 38 (Continued)

| Salinity | 20°C | | | | | |
|----------|-----------------|-----------------|-----------------|----------------|----------------|----------------|
| | Time (secs) | | | | | |
| | 5 | 10 | 20 | 40 | 60 | 120 |
| 0% | 6452.3 ***** | 2477.1 ***** | 1448.1 ***** | 212.3 ***** | 77.54 ***** | 23.23 ***** |
| 50% | 202.5 ***** | 286.5 ***** | 128.8 ***** | 50.07 ***** | 81.00 ***** | 273.8 ***** |
| 100% | 20.42 ***** | 68.60 ***** | 63.33 ***** | 48.62 ***** | 30.38 ***** | 7.336 * |

***** = $P < 0.001$;

**** = $0.005 > P > 0.001$;

** = $0.025 > P > 0.01$;

* = $0.05 > P > 0.025$.

(b) Second series of anovars - salinity effects

In the second series of analyses, Factor A was the three different salinities and Factor B the six time intervals. This resulted in six two-way anovars. Results of these are shown in Appendix II, Tables 29-34, pp. 486 - 491). Highly significant interactions were found in five anovars ($P < 0.001$). The interaction was not significant with the wet sediment at 10°C ($0.25 > P > 0.10$) (Appendix II, Table 30, pp. 487).

Because of the significant interactions, a series of breakdown one-way analysis were carried out comparing the three different salinities at the three different temperatures, at the six time intervals, and for wet and dry sediments. This resulted in thirty-six 1×3 one-way anovars (Appendix II, Tables 35-40, pp. 492 - 497). The F-ratios from these one-way anovars are shown in Table 39.

The results of these analyses were highly significant in thirty-two anovars out of thirty-six ($P < 0.001$). Significant differences were found in the other four anovars. These statistical analysis showed that there were highly significant differences in sedimentation produced by varying the salinity. Sedimentation was slower at higher salinities.

TABLE 39. Statistical comparisons of the effects of the three different salinities on sedimentation (mg/ml) F-ratios from thirty-six 1 x 3 one-way anovars (Appendix II, Tables 35-40; pp. 492-497).

| Temperatures (°C) | Wet | | | | | | Dry | | | | | |
|----------------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| | Time (secs) | | | | | | Time (secs) | | | | | |
| | 5 | 10 | 20 | 40 | 60 | 120 | 5 | 10 | 20 | 40 | 60 | 120 |
| 5 | 35.93 ***** | 353.5 ***** | 123.8 ***** | 8.393 *** | 12.17 *** | 131.5 ***** | 54.51 ***** | 175.4 ***** | 115.6 ***** | 75.26 ***** | 300.6 ***** | 173.6 ***** |
| 10 | 35.16 ***** | 15.26 ***** | 26.25 ***** | 55.12 ***** | 22.81 ***** | 15.57 ***** | 43.09 ***** | 9.062 **** | 57.08 ***** | 57.84 ***** | 15.53 ***** | 26.31 ***** |
| 20 | 32.92 ***** | 114.6 ***** | 190.0 ***** | 82.73 ***** | 179.6 ***** | 6.008 ** | 1762.1 ***** | 351.8 ***** | 154.1 ***** | 78.22 ***** | 347.8 ***** | 16.80 ***** |

***** = $P < 0.001$; **** = $0.005 > P > 0.001$; *** = $0.01 > P > 0.005$; ** = $0.025 > P > 0.01$

(c) Third series of anovars - temperature effects

In the third series of two-way anovars, Factor A was the three different temperatures and Factor B was the six time intervals. These anovars were carried out for each of the wet and dry sediments at the three different salinities. This resulted in six two-way anovars. Results are shown in Appendix II, Tables 41-46, pp. 498 - 503. Highly significant interaction factors were found in five anovars (Appendix II, Tables 42-46, pp. 499 - 503). The interaction factor was significant ($0.05 > P > 0.025$) in one anovar (Appendix II, Table 41, p. 498).

Because of the significant interactions, a series of breakdown one-way anovars were carried out comparing the three different temperatures for wet and dry sediment at the three different salinities, and at the six time intervals. This resulted in thirty-six anovars (Appendix II, Tables 47-52, pp. 504 - 509).

The F-ratios from these one-way anovars are shown in Table 40. Highly significant differences were found in thirty-one anovars ($P < 0.001$) and significant differences in three anovars. These statistical analyses show that the three temperatures have different effects on sedimentation. In general, sedimentation was fastest at 20°C , intermediate at 5°C , and slowest at 10°C (Figures 31-33).

TABLE 40. Statistical comparisons of the effects of the three different temperatures on sedimentation (mg/ml). F-ratios from thirty-six 1 x 3 one-way anovars (Appendix II, Tables 46-51, pp. 503 - 508).

| Salinity | Wet | | | | | | Dry | | | | | |
|----------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | Time (secs) | | | | | | Time (secs) | | | | | |
| | 5 | 10 | 20 | 40 | 60 | 120 | 5 | 10 | 20 | 40 | 60 | 120 |
| 0% | 81.28 ***** | 5.549 ** | 47.79 ***** | 76.35 ***** | 134.9 ***** | 53.90 ***** | 114.9 ***** | 11.48 **** | 24.61 ***** | 66.06 ***** | 58.61 ***** | 0 |
| 50% | 15.68 ***** | 24.99 ***** | 84.11 ***** | 16.81 ***** | 243.4 ***** | 38.79 ***** | 45.20 ***** | 10.92 **** | 57.36 ***** | 81.07 ***** | 0.3913 | 73.32 ***** |
| 100% | 32.95 ***** | 19.84 ***** | 32.92 ***** | 102.8 ***** | 25.97 ***** | 18.93 ***** | 26.78 ***** | 25.72 ***** | 538.9 ***** | 42.73 ***** | 32.90 ***** | 225.6 ***** |

***** = $P < 0.001$; **** = $0.005 > P > 0.001$; ** = $0.025 > P > 0.01$

The last series of breakdown one-way anovars tested differences between the six time intervals for wet and dry sediments at the three different salinities and the three different temperatures. This resulted in eighteen one-way anovars, six for each temperature (Appendix II, Tables 53, 54 and 55, pp. 510, 511, 512). Results from these analyses were all highly significant ($P < 0.001$) and show that the suspended weights decrease with time. This is obvious from Figures 31 to 33, and is, of course, to be expected.

4. Application of linear regression analysis to results

Line fitting curves

Three different equations were applied to the data where $x =$ time (secs), $y =$ suspended weight (mg/ml) to determine the best fitting one. These three equations were $y = a + bx$, $y = ae^{bx}$, and $y = ax^b$ (Appendix II, Table 56, p. 513). The three equations were ranked in terms of best fit, using the R values ($R =$ correlation coefficient). Based on this ranking the line equation $y = ae^{bx}$ was selected as giving the best fit (Table 41).

$y = ae^{bx}$ can be transformed to a straight line as follows:

$$\ln y = \ln (ae^{bx});$$

$$\ln y = \ln a + \ln (e^{bx});$$

$$\ln y = \ln a + bx.$$

This equation is equivalent to a straight line of the form $y = c + mx$. Hence a straight line is obtained by plotting $\ln y$ on the y-axis and time (x) on the x-axis (see Section III, p. 321).

The original data for suspended weights (mg dry sediment/ml) (Appendix II, Table 10, p. 467), were therefore transformed to

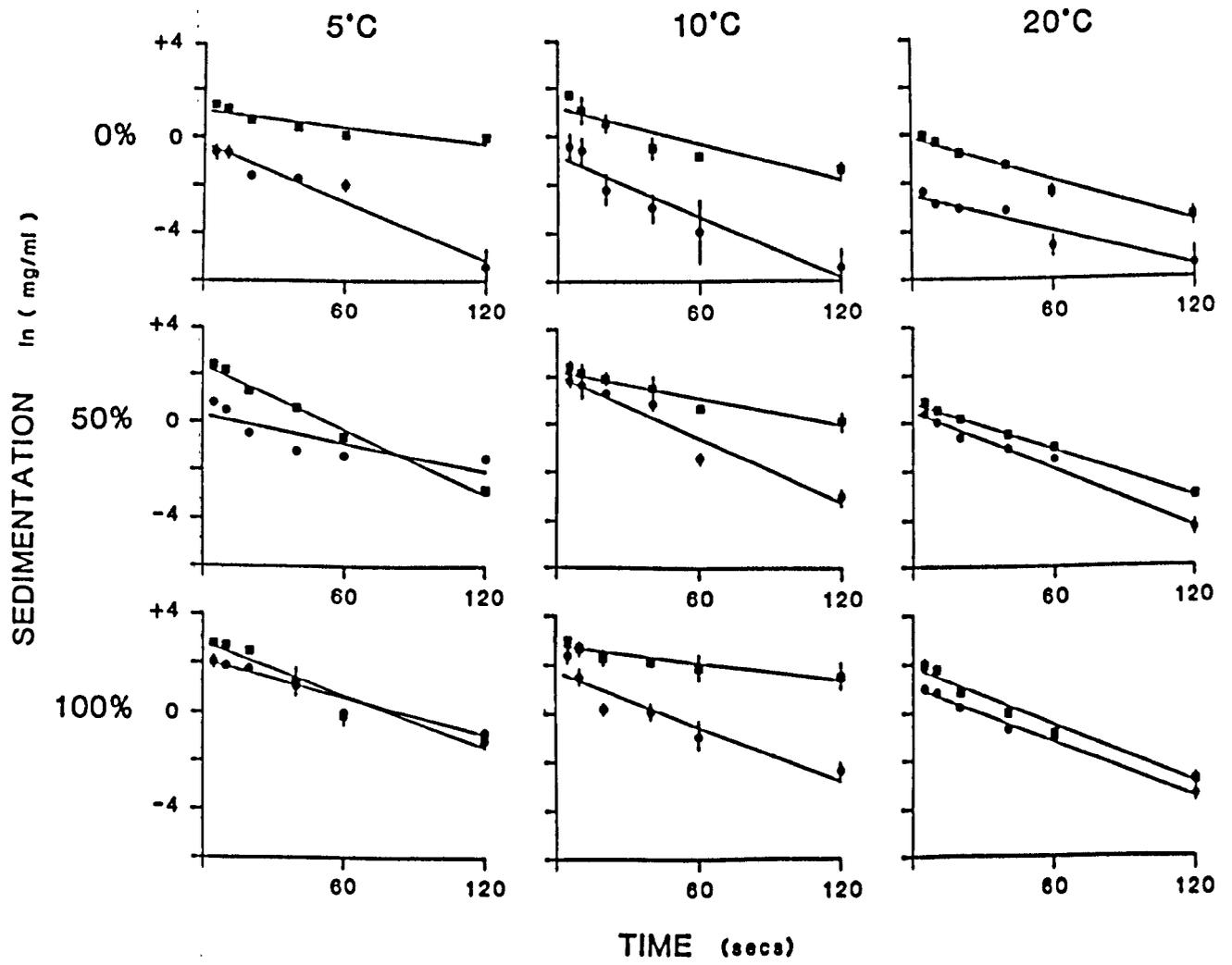
ln values. Units of the transformed data are therefore ln (mg dry sediment/ml). Regression analysis were then applied to the transformed data to give two (wet/dry) x 3 (three salinities) x 3 (three temperatures) = eighteen regression equations. These regression lines are shown in Figure 34, and their regression equations are shown in Tables 42, 43 and 44 for 5°C, 10°C and 20°C respectively. They are the linear equivalents to the curves plotted in Figures 31-33, and, of course, show the same differences.

TABLE 41. Langbank sediment. Ranking of the R values from the three different equations applied to the suspended weights obtained during sedimentation at three different temperatures, three different salinities, and wet and dry sediments (data in Appendix II, Table 56, p. 513.

| Rank | Number of times rank observed | | |
|------|-------------------------------|---------------|------------|
| | $y = a + bx$ | $y = ae^{bx}$ | $y = ax^b$ |
| 1 | 0 | 12 | 6 |
| 2 | 0 | 6 | 12 |
| 3 | 18 | 0 | 0 |

Figure 34

Relationship between \ln (mg dry weight sediment/ml) =
 y and time (secs) = x , for wet (■—■) and dry
(●—●) Langbank sediment at three different temperatures
(5°C, 10°C and 20°C) and at three different salinities
(0%, 50% and 100%). Points represent means and
standard deviations of five replicate readings. Some
standard deviations were so small that they cannot be
seen. The original untransformed data (mg dry sediment/
ml), are given in Appendix II, Table 10, p. 467.



The correlation coefficient (r) values from the linear regression analyses (Tables 42, 43 and 44) were used to calculate t values using the following equation:

$$t = \frac{r \sqrt{n-2}}{\sqrt{1-r^2}} \quad (\text{d.f.} = n-2)$$

(Bailey, 1959, p. 85; Moroney, 1951, p. 311; Rohlf and Sokal, 1969, p. 224).

All the t tests were highly significant ($P < 0.001$) (Tables 42, 43 and 44). This proves the obvious fact that suspended weights decreased with time for all the curves.

Table 45 shows two parameters that can be predicted from the regression equations - the weight of sediment at 5 seconds as a percentage of the initial added weight, and the time at which 0.1% of the sediment remains in suspension. The two parameters broadly confirm the previous statistical analyses: sedimentation occurs more quickly when the sediment is previously dried, sedimentation occurs more slowly at higher salinities, and sedimentation usually occurs more quickly at 20°C than at 5°C or 10°C.

One interesting fact becomes apparent from the values of the present suspended weights at 5 seconds. In these experiments a very large proportion of the sediment had fallen below the 10 cm level by the time the first (5 seconds) reading was taken. The percentages range from 17.2% to 0.1%. In other words, 82.8% to 99.9% has fallen below the 10 cm level within 5 seconds. There is no way of sampling in a shorter time period with my technique.

TABLE 42. Linear regression analyses of the suspended weight $y = \ln$ (mg dry sediment/ml), against time (secs) of the wet and dry Langbank sediment at the three different salinities. 5°C data.

| Temperature | Sediment type | Salinity | Equation $\ln y = \ln a + bx$ | Correlation coefficient (r) | T | Probability |
|-------------|---------------|----------|----------------------------------|--------------------------------|-------|----------------------|
| 5°C | Wet | 0% | $\ln y = 1.108 - 0.01185X$ | -0.8589 | 8.873 | $P < 0.001$ ***** |
| | | 50% | $\ln y = 2.414 - 0.04525X$ | -0.9907 | 38.55 | $P < 0.001$ ***** |
| | | 100% | $\ln y = 2.869 - 0.03692X$ | -0.9369 | 14.18 | $P < 0.001$ ***** |
| | Dry | 0% | $\ln y = -0.3086 - 0.03962X$ | -0.9507 | 16.22 | $P < 0.001$ ***** |
| | | 50% | $\ln y = 0.2408 - 0.01909X$ | -0.8035 | 7.143 | $P < 0.001$ ***** |
| | | 100% | $\ln y = 2.136 - 0.02654X$ | -0.9617 | 5.292 | $P < 0.001$ ***** |

TABLE 43. Linear regression analyses of the suspended weight $y = \ln$ (mg dry sediment/ml), against time (secs) of the wet and dry Langbank sediment at the three different salinities. 10°C data.

| Temperature | Sediment type | Salinity | Equation $\ln y = \ln a + bx$ | Correlation coefficient (r) | T | Probability (P) |
|-------------|---------------|----------|----------------------------------|--------------------------------|-------|----------------------|
| 10°C | Wet | 0% | $\ln y = 1.177 - 0.02503X$ | -0.8714 | 9.399 | $P < 0.001$ ***** |
| | | 50% | $\ln y = 2.345 - 0.02016X$ | -0.9013 | 11.01 | $P < 0.001$ ***** |
| | | 100% | $\ln y = 2.788 - 0.0111X$ | -0.7531 | 6.056 | $P < 0.001$ ***** |
| | Dry | 0% | $\ln y = -0.7981 - 0.04127X$ | -0.8706 | 9.364 | $P < 0.001$ ***** |
| | | 50% | $\ln y = 2.112 - 0.04409X$ | -0.9603 | 18.22 | $P < 0.001$ ***** |
| | | 100% | $\ln y = 1.702 - 0.03648X$ | -0.9201 | 12.43 | $P < 0.001$ ***** |

TABLE 44. Linear regression analyses of the suspended weight $y = \ln$ (mg dry sediment/ml), against time (secs) of the wet and dry Langbank sediment at the three different salinities. 20°C data.

| Temperature | Sediment type | Salinity | Equation $\ln y = \ln a + bx$ | Correlation coefficient (r) | T | Probability (P) |
|-------------|---------------|----------|----------------------------------|--------------------------------|-------|----------------------|
| 20°C | Wet | 0% | $\ln y = -0.08059 - 0.02979X$ | -0.9704 | 21.26 | $P < 0.001$ ***** |
| | | 50% | $\ln y = 0.8594 - 0.03239X$ | -0.9956 | 56.23 | $P < 0.001$ ***** |
| | | 100% | $\ln y = 1.902 - 0.04053X$ | -0.9800 | 26.06 | $P < 0.001$ ***** |
| | Dry | 0% | $\ln y = -2.519 - 0.02538X$ | -0.9113 | 11.71 | $P < 0.001$ ***** |
| | | 50% | $\ln y = 0.4708 - 0.03958X$ | -0.9885 | 34.57 | $P < 0.001$ ***** |
| | | 100% | $\ln y = 1.070 - 0.03757X$ | -0.9887 | 34.88 | $P < 0.001$ ***** |

TABLE 45. Suspended weight (mg/ml) at 5 seconds of sedimentation as a percentage of the initial sediment weights and the time of 99.9% sedimentation at the three different temperatures, three different salinities and for wet and dry sediments.

A: suspended weight at 5 seconds predicted from regression equation as a % of initial weight.

B: time (secs) predicted from regression equations at which 0.1% of sediment remains in suspension, i.e. 99.9% sedimentation.

| Salinity | Sediment type | 5°C | | 10°C | | 20°C | |
|----------|---------------|-------|------------------|-------|------------------|------|------------------|
| | | A | B | A | B | A | B |
| 0% | Wet | 3.4% | 301 _s | 3.35% | 145 _s | 0.9% | 80 _s |
| | Dry | 0.7% | 70 _s | 0.4% | 79 _s | 0.1% | < 5 _s |
| 50% | Wet | 10.5% | 108 _s | 11.1% | 239 _s | 2.4% | 103 _s |
| | Dry | 1.4% | 142 _s | 7.8% | 104 _s | 1.5% | 74 _s |
| 100% | Wet | 17.2% | 143 _s | 18% | 473 _s | 6.4% | 108 _s |
| | Dry | 8.7% | 173 _s | 5.4% | 114 _s | 2.8% | 94 _s |

SECTION II

Discussion

Sedimentation of sediment particles has been investigated in a number of studies (see Section II, Introduction, p.179 for references). The above studies were conducted on different aspects of the sedimentation process. They are concerned with theories, hydrodynamics, particle sizes and some physical and biological effects during the sedimentation process.

Three papers are directly related to the work in this section. Krank (1975) studied sediment deposition from flocculated suspensions. He found that suspended sediments in coastal environments with high inorganic content have characteristically broad size distributions and are composed of single grains and flocculated aggregates. Krank compared the theoretical settling speed of quartz grains with the settling speed of particles in natural suspensions under experimental conditions. This comparison indicated that most grains smaller than the deflocculated single grain mode settle as part of flocs, whereas particles larger than the mode settle as single grains.

The second paper is the work of Peirce and Williams (1966) on certain aspects of sedimentation of estuarine muds. He found that sedimentation processes are different for different estuarine muds. At high salinities, contacts between particles produce flocs, and the presence of coarse non-colloidal particles will affect floc formation. His experiments also showed that at low salinities separation occurs between fine and coarse material (differential settling). Above a certain solids concentration - which was different for each mud - floc networks of different sizes are formed. Peirce and Williams (1966) experiments also demonstrated that when muds are permitted to settle, they compact under their own weight to different extents.

The last paper is that of Postma (1967) on sediment transport and sedimentation in the estuarine environment. He presented a review of sediment movement in nearshore waters, tidal flat areas, and estuaries. Postma (1967) states that various transport mechanisms may be active in holding the material in these regions. Among the accumulation mechanisms holding suspended matter near the coast are settling and scour lag effects which in combination with tidal movements can lead to residual transport towards the coast. Density differences, especially those occurring in and near rivers may result in the concentration of suspended materials in turbidity maxima. Postma (1967) states that the confinement of suspended matter within a certain region, combined with movement by tidal and density currents, has an important selective effect on the grain size distribution of the deposits.

The remainder of the Discussion in this section is divided into two parts. The first discusses how sedimentation is influenced by organic material attached to particles, and the second discusses the effects of temperature, salinity and drying on sedimentation.

(I) Influence of organic material attached to sediment particles on sedimentation

Removing organic films from surfaces of sediment particles by ashing and acid-cleaning always increased sedimentation. This means that in the field, particles of a given size will settle more quickly if they have little organic material and microbial growth on them and more slowly if they have a lot of organic material and microbial growth.

There appear to be no field or experimental studies of this phenomenon, and the only related work which I could find was by Hunter and Liss (1979, 1982), Krank (1973) and Webb (1969).

Hunter and Liss (1979) studied the surface charge of suspended particles in estuarine and coastal water. They stated that their

results provided the first direct evidence of a dominant role of oxide and/or organic films in determining the surface properties of suspended estuarine particles. Hunter and Liss (1979) also reported that in previous work similar methods had established that natural organic surfactants largely control the properties of surfaces introduced into sea water through the formation of cohesive films. Hunter and Liss (1979) feel that the basic principles revealed in their study are sufficiently general to apply in other areas. For example, they stated that the uniformity of electrokinetic charge on suspended estuarine particulates arising from the dominant presence of surface coatings, may explain the apparent lack of evidence for differential flocculation of minerals in field studies. Furthermore, they state that surface films are likely to exercise a major role in chemical transformations involving particulate matter. Thus, for example, uptake or release of dissolved trace metals by particles may well depend mainly on the chemistry of the organic matter forming the film rather than on the nature of the underlying solid matrix.

Flocculation of suspended sediment in the sea is a complicated phenomenon (see Introduction, p. 152). Flocculation of particles depends on two factors: particle collision during transport and adhesion of particles on contact (Hahn and Stumm, 1970; Packham, 1962). Krank (1973) stated that particles of different sizes will respond differently to gravity and turbulent forces. The smaller particles will flocculate most rapidly, because of their relatively larger surface area and proportionally greater adhesive force (Van Olphen, 1966). Larger grains may not be sufficiently surface active to flocculate with other single grains, but they do adhere to the soft irregularly shaped flocs composed of many smaller grains (Krank, 1973). Krank refers to earlier work showing that inorganic sediment suspended in the sea is unstable as single mineral particles, and occurs as

flocculated aggregates with settling speeds many times greater than that of the constituent grains; this was demonstrated in the field and by laboratory experiments.

Krank (loc. cit.) studied flocculation in natural samples of sea water by membrane filtration followed by ashing the membrane filter to remove the organic material. Under the microscope flocs appear as irregular masses or clumps of numerous individual particles. He showed that there was a highly significant positive correlation between the size of the flocs and the size of the individual particles within the flocs. This correlation was based on modal particle sizes ranging between about $0.5\ \mu$ and $64\ \mu$ and on modal floc sizes between about $2\ \mu$ and $64\ \mu$. Most of the aggregates consisted of smooth transparent sub-rounded mineral grains. The flocs form the largest particles in all samples but also range down to minute aggregates of the smallest grains. They are composed of an unsorted mixture of large and small grains. Individual grains not forming part of flocs also occurred. These were more abundant among the larger grains and the largest grains in a distribution never seen to become part of floc. Krank (1973) showed that when gently shaken, the bigger flocs move about and consist of a relatively low density soft jelly-like loose network of grains. He concluded that in sea water particles flocculate into characteristic stable size distributions, the modes of which are determined by the initial size of the constituent grains. Krank also conducted laboratory experiments which mimicked the effect observed under natural conditions.

The last example is work by Webb (1969) on biologically significant properties of submerged marine sands. Webb (1969) considered that bacterial films can change the properties of sand by altering the adhesiveness of particles and by increasing their size and uniformity of shape, thereby modifying the interstitial lattice work.

My results showed that removing organic films from surfaces of sediment particles always increased sedimentation (Figures 28, 29 and 30, pp. 217, 219, 221 respectively). I therefore decided to investigate the relationship between organic content (Section I, Table 13, p. 98) and suspended dry weight obtained during the sedimentation experiments (Section II, Table 28, p. 215). Six regression lines were constructed for the six time intervals (5, 10, 20, 40, 60 and 120 seconds).

These regression lines show two major points (Figure 35). Firstly, there was a close linear relationship between the amount of organic material on sediment particles and suspended weight. Secondly, the slopes of the regression lines increased with increasing time - except at 120 seconds. The equation of each of the above lines and their correlation coefficients (R) and students' t values are shown in Table 46. All the t values were highly significant ($P < 0.001$), thus proving that all the slopes were significant.

I then decided to investigate the relationship between the slopes of the regression lines and particle size obtained in an experiment on Rockware sediment in the next section (Appendix III, Table 30, p. 546, Figure 36). This can only be an approximate comparison because the regression lines and hence their slopes were obtained from Langbank, Ardmore and Rockware sediments, while the particle size was obtained from only one of these - Rockware sediment. However, the particle size distribution of the three sediments are broadly similar, so the comparison is a realistic one. Figure 36 shows that the slope of the line increases as the particle size decreases to c. $70 \mu\text{m}$, and then falls as the particle size decreases further to $62 \mu\text{m}$ (120 seconds). (For details of how data were plotted see legend to Figure 36.) This means that the effect of organic material on the smaller particles is greater than on the larger ones down to a particle size of about $70 \mu\text{m}$,

probably because smaller particles are covered with more organic material per unit volume. However, it is not clear why this relationship does hold below c. 70 μm ; perhaps particles smaller than 70 μm have less organic material/unit surface area.

An inverse relationship between particle size and percentage organic content has been shown by Longbottom (1970, Figures 3 and 4, p. 144). From Longbottom's figures, it is clear that organic nitrogen and organic carbon both vary logarithmically with median particle diameter. Longbottom (1970) showed that the highest amounts of organic matter are found in the finest deposits. In general, the results in my study are similar to the results of Longbottom (1970) showing an increase in the amount of organic matter with decreasing particle size - with an exception for the particle sizes obtained at 120 seconds. The smallest particle sizes shown in Longbottom's study were c. 90 μm , while the mean particle size I measured at 120 seconds was c. 62 μm . I am therefore unable to relate my result at 120 seconds to Longbottom's work.

The effect of organic material on sedimentation can be interpreted in terms of Stokes' Law:

$$v = \frac{2(d_s - d_f)gr^2}{9\mu}$$

Settling velocity (V) is directly related to the difference between the density of the settling particles (d_s) and that of the medium (d_f). Removing organic material from sediment particles means removing most of the low density material. Since sedimentation is affected by the difference between d_s and d_f , removing organic material produces an increase in the difference between d_s and d_f , which in turn increases settling velocity (V). In other words, the greater the difference between d_s and d_f the faster the sedimentation, and the smaller the difference the slower the sedimentation.

Finally, it is possible that factors such as surface charge and flocculation may have some effect on sedimentation (Hunter and Liss, 1979; Krank, 1973; Neihof and Loeb, 1972; Pravdic, 1970), but it is not clear how this would work or whether the effects would be at all significant. I did not measure surface charge, but I saw no obvious evidence of flocculation in any of my experiments.

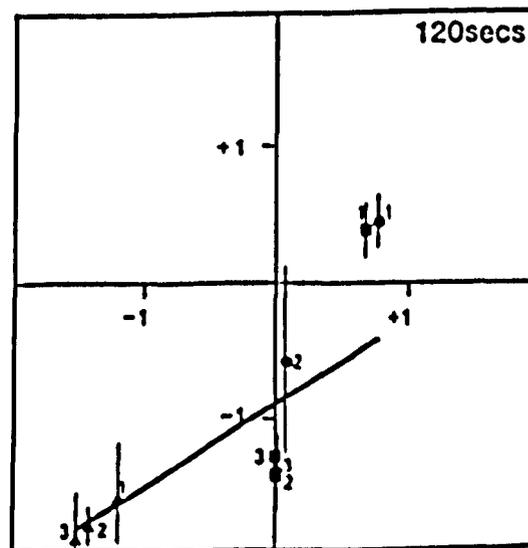
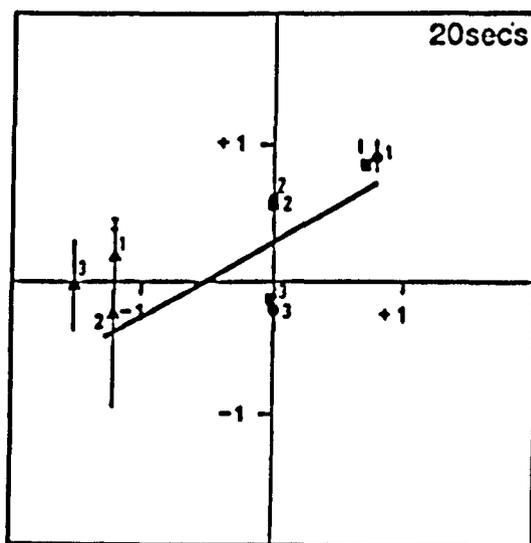
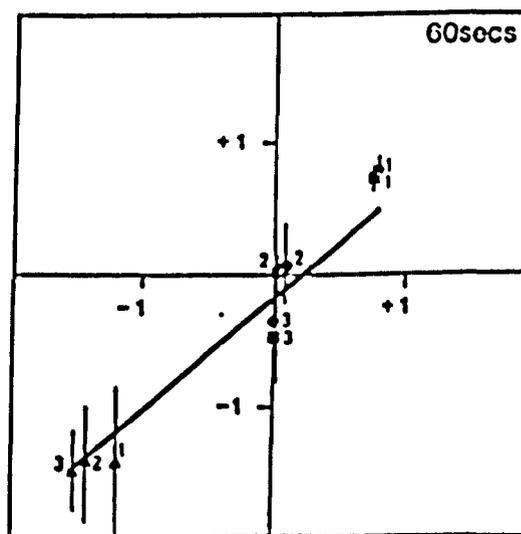
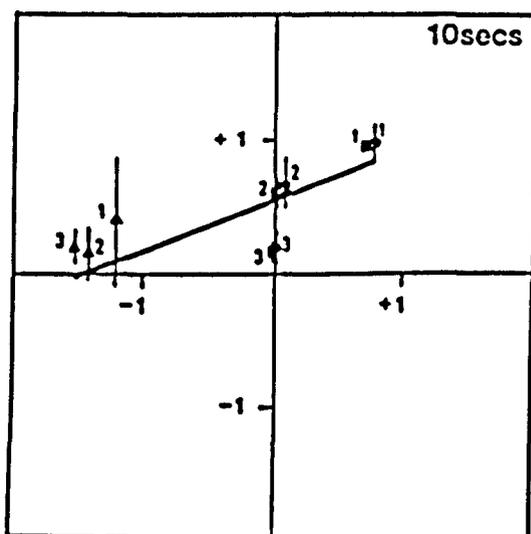
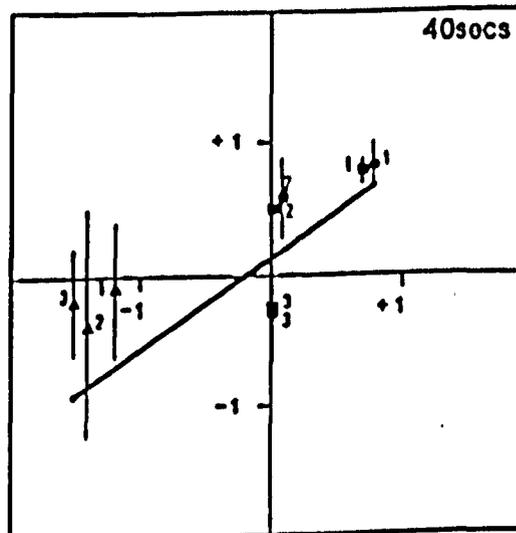
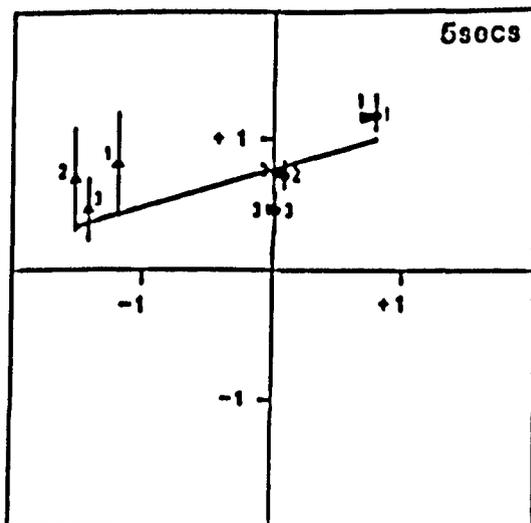
The field implications of this part of my work are important. The more organic material present on sediment particles, the longer time these particles remain in suspension. Therefore, particles having a lot of organic material on their surfaces will be carried further by water currents than cleaner particles. In estuaries where the main movement of sedimentary material is from the river to the sea, particles with rich organic coatings will therefore sediment further down the estuary than cleaner particles.

It would be entirely feasible to test this hypothesis in an estuary by taking samples of sediments down the estuary from the river to the sea. These would then be sieved to give different particle size fractions, and the organic content of each fraction would be measured. The hypothesis will be substantiated if for a given size fraction, organic carbon increases down the estuary towards the sea. In my study area, the Clyde Estuary, this general trend might be confused by local sewage discharge points such as that at Langbank. For example, I have shown that Langbank sediment has more pollution than Ardmore sediment which is situated further down the estuary (Section I, p. 32). However, my hypothesis could be true in an estuary with no human habitation or activities close to it.

Figure 35

Relationship between organic content ($\log_{10} (\text{mg C.g}^{-1})$) = X and suspended weight ($\log_{10}(\text{mg/ml})$) during sedimentation = y for the control (1), acid-cleaned (2) and ashed (3) of Langbank (●), Ardmore (■) and Rockware (▲) sediments. Points represent means and standard deviations of ten readings. Some standard deviations were so small that they cannot be seen.

SUSPENDED WEIGHT $\log_{10} (\text{mg} \cdot \text{ml}^{-1})$



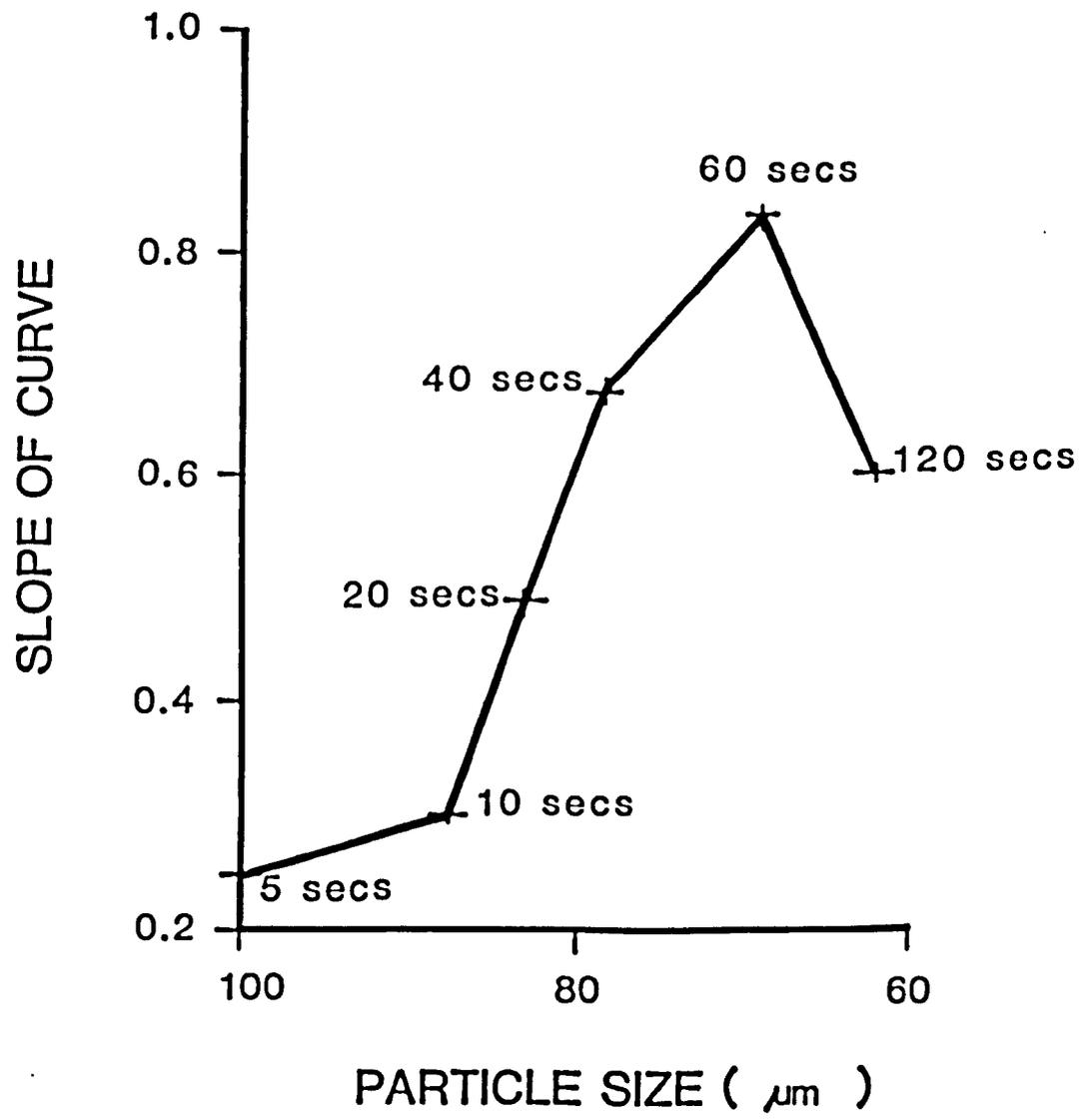
ORGANIC CARBON $\log_{10} (\text{mg C} \cdot \text{g}^{-1})$

TABLE 46. Linear regression analyses between the organic content ($\log \text{ mg C.g}^{-1}$) and suspended dry weight sediment ($\log \text{ mg ml}^{-1}$) obtained during sedimentation of control, ashed and acid-cleaned sediments for Langbank, Ardmore and Rockware sediments, at six time intervals.

| Time (secs) | $y = A + BX$ | R | T (B = 0) | D.F | Probability |
|-------------|-------------------------|--------|-----------|-----|----------------------|
| 5 | $y = 0.7455 + 0.2586X$ | 0.5469 | 6.129 | 90 | $P < 0.001$ ***** |
| 10 | $y = 0.4949 + 0.3271X$ | 0.5810 | 6.697 | 90 | $P < 0.001$ ***** |
| 20 | $y = 0.3302 + 0.4919X$ | 0.6673 | 8.406 | 90 | $P < 0.001$ ***** |
| 40 | $y = 0.1222 + 0.6774X$ | 0.7127 | 9.531 | 90 | $P < 0.001$ ***** |
| 60 | $y = -0.1781 + 0.8338X$ | 0.7384 | 10.27 | 90 | $P < 0.001$ ***** |
| 120 | $y = -0.6037 + 0.9073X$ | 0.5786 | 6.655 | 90 | $P < 0.001$ ***** |

Figure 36

Relationship between the slopes of the regression lines of suspended weight against organic content ($\text{mg sed.ml}^{-1} / \text{mg C.g}^{-1} \text{ sed}$) (Table 46, p. 269) = y , and particle size (μm) = x from the refined sedimentation experiment on Rockware sediment (Appendix III, Table 30, p. 546). Horizontal lines represent the standard error of the mean particle sizes. Vertical lines represent the standard error of the regression lines.



(II) Effects of temperature, salinity and drying on sedimentation

The results of this part can be summarised as follows.

Wet sediment settles much more slowly than dry. The three different temperatures produced different effects on sedimentation. The most rapid sedimentation occurred at a temperature of 20°C. Sedimentation at 10°C and 5°C were similar but was slightly slower at 10°C.

Salinity also had a significant effect. The most rapid sedimentation occurred at 0% salinity and the lowest at 100%.

The discussion of this part is divided into two subdivisions. The first describes the effects of drying on sedimentation. The second describes the effects of temperature and salinity on sedimentation.

1. Effects of drying on sedimentation

Differences in the sedimentation of wet and dry sediments may be explained as follows.

Sediment particles are covered with a film of different types of microorganisms and organic material (see Section I, Introduction, p.25 for references). Drying probably causes a disruption of these films which is great enough to influence their sedimentation. This may occur by the film contracting onto the surface thus making the particle effectively more dense. It will therefore sediment more quickly. I can find no references in the literature to similar work.

2. Effects of temperature and salinity on sedimentation

The density of sea water increases with salt concentration and decreases with temperature (Meadows and Campbell, 1978). Hence one would in general expect that sedimentation would be slower at higher salinities and faster at higher temperatures. This broadly agrees

with published work and the results presented in this section of my thesis.

The effects of temperature on sedimentation have been studied by several authors (Gibbs et al., 1971; Perkins, 1974).

Gibbs et al. (1971) studied the settling velocities of glass spheres ranging in size from 50 μ to 5000 μ . They measured settling velocities at six different temperatures, 5, 10, 18, 20, 22 and 30°C. They found that settling velocity increased with particle diameter and that the settling velocities of all the glass spheres increased as the temperature increased. In my experiments, particles from Langbank sediment settled more quickly at 20°C than at 5 or at 10°C although the settlement of particles at 10°C was slower than that at 5°C. Hence my results do not agree entirely with those of Gibbs et al. (1971). The reason for this is not apparent.

The effects of salinity on sedimentation can broadly be predicted from Stokes' Law since the settling velocity is directly related to the difference between the density of the settling particles and that of the medium. For a particle at a given density, the higher the density of the medium the slower the particle will settle. Water of a progressively higher salinity has a progressively higher density and so sediment particles will sediment more slowly in it. This is precisely what I observed in my experiments: particles settled more slowly at a higher salinity.

Settling velocity (V) is also directly related to the viscosity of the medium (μ). Temperature affects the viscosity of the medium (μ) as well as its density (d_f): the higher the temperature, the smaller the values of both density and viscosity and hence the faster the sedimentation.

In my experiments sedimentation was faster at 20°C than at 10 or 5°C, but slower at 10°C than at 5°C. I am unable to explain this latter observation.

(III) Hindered settling

All my sedimentation experiments were conducted with relatively high concentrations suspended solids (sediment) of 85 g/litre. Hindered settlement (see Introduction, p.164 for details) occurs at a suspension of greater than about 2 g/litre. Therefore all my experiments have been conducted in the hindered settlement regime. In this regime there is a significant upward displacement of water due to the settling particles and this has the effect of reducing the apparent settling velocity of the particles.

The theory of hindered settling is extremely complex (Allen, 1975) and Stokes' Law may not be strictly applicable (Galehouse, 1971). However, the obvious effects that I have demonstrated of temperature, salinity, removal of organic material and animal secretions (see Section III) all have important implications for the settlement of sedimentary particles in the field.

SECTION II

Summary1. Preliminary experiments

Preliminary sedimentation experiments were conducted to find the best sampling time and volume.

The following times and volumes were tested: 5, 10, 20, 40, 60 and 120 seconds, 5, 10, 20, 40, 60 minutes and 24 hours; 1, 2, 4, 6 and 8 ml. The largest weight of sediment (mg/ml) was obtained in the 2 ml sample.

A definitive method was developed, based on the results from the preliminary experiments which was as follows.

42.61 \pm 1.301 g of dried sediment (\bar{x} 55.36 g wet sediment) and 500 ml of synthetic sea water were placed in a 500 ml stoppered glass measuring cylinder. The cylinder was inverted three times before taking any sample. Two millilitre pipette samples at 10 cm depth were removed at 5, 10, 20, 40, 60 and 120 seconds.

The preliminary experiments were carried out using Langbank and Rockware sediments only.

2. Effects of organic material on sedimentation(a) Measuring and staining of organic material on particles

Two methods were used to remove organic material from Langbank, Ardmore and Rockware particles: ashing and acid-cleaning.

Particles were stained to visualise organic material. The amount of stained materials on Langbank and Ardmore sediment particles were much higher than on Rockware particles.

Both cleaning methods reduced the stained material (i.e. organic material) on the three sediments. The amount of stained material on sediment particles agreed with the organic carbon measurements before and after cleaning.

(b) Influence of organic material on sedimentation

Langbank, Ardmore and Rockware sediments were cleaned by acid-cleaning and ashing (thus removing different amounts of organic material). Removing organic material from particles had a profound effect on sedimentation: particles with less organic material on them always settled more quickly.

A linear relationship was found between Log_{10} (organic carbon) (x) and Log_{10} (suspended sediment) (y). This effect increased with time (the slopes of the linear regression increased), except at 120 seconds, and is probably caused by the decreasing particle size of particles in suspension as time increases.

3. Effect of dryness, temperature and salinity on sedimentation

(a) Dryness

Wet sediments settled much more slowly than dry ones. Results were all highly significant statistically.

(b) Temperature

Sedimentation occurred most rapidly at 20°C. Sedimentation at 10°C was slightly slower than at 5°C.

(c) Salinity

Sedimentation occurred most rapidly at 0% salinity, and most slowly at 100% salinity (35‰).

The suspended weights at 5 seconds as a percentage of initial weight were predicted from the regression equations. These values were calculated for the three different salinities (0%, 50% and 100%) ^(0‰, 17.5‰, 35‰) and for the three different temperatures (5, 10 and 20°C).

The percentage values for the wet sediment at the three different salinities (0%, 50% and 100%) were (3.4, 10.5, 17.2%), (3.35, 11.1, 18%) and (0.9, 2.4, 6.4%) at the 5, 10 and 20°C respectively. For the dry sediment at the above three salinities, these percentages were (0.7, 1.4, 8.7%), (0.4, 7.8, 5.4%) and (0.1, 1.5, 2.8%) for the 5, 10, 20°C respectively.

4. Stokes' Law

The effect of organic material, salinity and temperature were interpreted in terms of Stokes' Law.

5. Field implications

The effect of organic material on sedimentation and its field implications is discussed.

SECTION III

THE EFFECTS OF ANIMAL SECRETIONS ON SEDIMENTATION

SECTION III

Introduction

Many workers have shown that the activity of benthic microorganisms, plants and animals modify the physical and chemical nature of marine sediments.

The effects of microorganisms (I) and plants (II) on their sedimentary environment will be reviewed briefly below. Emphasis will be given to the effect of benthic animals (i.e. invertebrates) on marine sediments, since this forms the subject of the present section. In the Introduction, I describe some work which is not closely related to my own. This is because I feel that a broad overview of the subject is required at this point.

(I) The effect of microorganisms

Microorganisms are believed to affect terrestrial soil properties mainly by their effects on the stability of soil aggregates (Aspiras et al., 1971; Martin and Waksman, 1940). During the degradation of biological remains by bacteria and fungi, polysaccharides and humic substances are formed. These substances stabilise soil aggregates by forming polymer bridges between individual soil particles (Hayes, 1980). Sediment binding can also occur when microbial growth structures such as fungal hyphae are formed between particles (Aspiras et al., 1971). Aspiras et al. (1971) found that the stability of soil aggregates was maintained by different chemical-binding agents produced in situ by pure cultures of microorganisms and by an indigenous soil microflora. They also found that seven fungi, six streptomycetes, and five bacteria produced binding materials in artificial aggregates of Miami silt loam, enriched with sucrose. The effect of microorganisms on terrestrial soil erodibility has been reviewed and discussed by Gasperi-Mago and Troeh (1979).

There are few studies on the effect of marine microorganisms on sediment stability. However, it is known that marine bacteria like their terrestrial counterparts, secrete polysaccharides for attachment to surfaces (Sutherland, 1980). These polysaccharides may also bind sediment particles together thereby increasing the strength of the sediment. The effect of microbial polysaccharides on the stabilisation of natural soil aggregates has been studied by a number of research workers (Barker et al., 1967; Browning and Milam, 1944; Geoghegan and Brian, 1946, 1948; Greenland et al., 1961; Haworth et al., 1946; Martin, 1945, 1946; Waksman and Martin, 1939). A study was conducted by Webb (1969) on the biologically significant properties of submerged marine sands. He showed that the extent and thickness of microbial films significantly changed the adhesiveness of grains and hence the geometry of the lattice they form. The films also change the size and shape of the individual particles.

(II) The effect of plants

The ability of terrestrial plants to modify their physical environment has been known for some time. The initial stabilisation of sand dunes by pioneer grasses is one of the best examples of the way in which plants can stabilise sediments (Odum, 1959).

Studies conducted in the marine environment indicate that marine algae may perform a similar function. Sediment stabilisation by marine algae generally occurs by one of three mechanisms.

1. Baffles

Dense colonies of sea grasses or benthic macro-algae reduce the velocity of bottom currents allowing fine sediment to settle out (Frostick and McCave, 1979; Ginsburg and Lowenstam, 1958).

2. Organic films

Micro-algae produce organic films on the sediment surface which increase adhesion between sediment particles and reduce resuspension of sediment (Bathurst, 1967; Black, 1933; Frankel and Mead, 1973; Holland et al., 1974).

3. Framework structures

Both macro- and micro-algae produce filaments in the sediment which act as a rigid supporting skeleton (Neumann et al., 1970; Scoffin, 1970).

(III) The effect of animals

Animal sediment interactions can be considered from two points of view: (1) the influence of sediment properties on the distribution of animals living in them (i.e. habitat selection) and (2) the modification of sediments by animal activity (i.e. bioturbation).

1. The influence of sediment properties on the distribution of animals

The sedimentary environment determines the types of organisms which may be present. It provides the optimum conditions for certain species and prevents the intrusion of others. There are a large number of studies on the effects of sediment parameters on animals. Early work by Meadows (1964a,b,c) on substrate selection by Corophium, followed by two major reviews of the field by Meadows and Campbell (1972a,b) have shown that benthic invertebrates select a suitable habitat by responding to a variety of physical, chemical and biological properties of sediments.

Since then, a number of papers have appeared analysing habitat selection in field and laboratory studies (Bloom et al., 1972; Cullen, 1973; Featherston and Risk, 1977; Johnson, 1971; Kendall, 1979;

Myers, 1972; Nichols, 1970; Sanders, 1958).

Bloom et al. (1972) studied animal sediment relations and community analysis of a Florida estuary. They found that numbers of deposit feeders were inversely correlated with filter feeders, and both trophic types were correlated to the sediment parameters of median grain size, sorting and skewness.

Featherstone and Risk (1977) studied the effect of tube-building polychaetes on intertidal sediments of the Minas Basin, Bay of Fundy. These mud flats support large populations of invertebrates whose zonation is markedly influenced by sediment type.

Diopatra cuprea (Polychaeta:Onuphidae) builds a long nearly vertical parchment tube in sediments, which is reinforced with bits of shell, sediment or debris (Myers, 1972). Its tube is reinforced at or above the sediment surface, and unreinforced below it. If sediment accumulates faster than the worm can build the reinforced section, it builds an unreinforced section.

2. The effect of animals on sediments

This topic has received increasing attention recently. Benthic animals can produce different effects on sediments. These effects include chemical and mechanical (i.e. transport, stabilise, destabilise) and may be classified as follows.

(a) Effect of benthic invertebrates on sediment mixing, transport and stability

Benthic invertebrates affect sediment transport and stability by reworking sediments during movement and feeding, and by burrow and tube building (Inderbitzen, 1974; McCall and Tevesz, 1982; Wiley, 1978).

Reworking

Reworking alters the spatial arrangement of sediment particles which in turn modifies other physical and chemical properties (Lee and Swartz, 1980), mixing and transporting sediment particles as well as interstitial water and gases (Gray, 1974; Kikuchi and Kurihara, 1977; Rhoads, 1963, 1967, 1974; Rhoads and Young, 1971).

Rhoads (1967) studied the biogenic reworking of intertidal and subtidal sediments by Clymenella torquata, Pectinaria gouldii and Amphitrite ornata in Barnstable Harbor and Buzzards Bay, Massachusetts. Selective ingestion and transportation of sand and smaller-sized particles leads to biogenically graded deposits. There were also differences in intensity of reworking which were related more to faunal composition than to faunal density. Extensive reworking produces a characteristically flat sea floor due to continuous resuspension and deposition of sediment (Rhoads, 1974).

Reworking often deposits layers of faecal pellets at the sediment-water interface. These pellets have a high water content and a low density and are easily eroded by tidal currents (Yoldia limatula (Rhoads, 1963); Clymenella torquata (Rhoads, 1967); Nucula proxima (Rhoads and Young, 1970)).

Reworking varies seasonally (Cadée, 1976; Grant et al., 1982; Nichols, 1974; Schäfer, 1972). The zone (depth) of sediment mixing caused by burrowing organisms depends on the type of burrowers. In oceanic sediments, the zone of mixing ranges from 10 to 30 cm with a mean value of 20 cm (Donahue, 1971). A relationship between reworking intensity and sediment organic content has also been noted (Gordon, 1966; Nichols, 1974).

Nichols' (1974) data suggested that there can be a clear seasonality in the rate of turnover. Warming of bottom water in summer may serve to increase turnover rates, especially as the large number of recently settled invertebrates begin to exert an influence on total biomass.

The amount of organic matter in sediments can affect sediment turnover rates (Gordon, 1966). A review of reworking by deposit feeders in estuaries and inshore sediments is given by Winston and Anderson (1971) and Cadée (1976).

Stability

If tubes and burrows of specific invertebrates are present in sufficient numbers they can increase the stability of the sediment. Fager (1964) found that a dense settlement of the tubicolous polychaete, Owenia fusiformis, stabilised a shifting sand against erosion. The tubes acted as a rigid supporting framework in the sediment. Extensive burrowing of deposit-feeding organisms affects the stability of reworked sediments (Young and Rhoads, 1971). Densely populated tube mats of tubicolous polychaetes in Cape Cod Bay, Massachusetts, U.S.A., bind and stabilise the substratum, providing solid surfaces for attachment of epizoans. Young and Rhoads (1971) found that the faecal mounds of the holothurian, Molpadia colitica, were stabilised by tubes of the small polychaete, Euchone incolor. Powell (1977) noted that burrows of the holothurian, Leptosynapta tenuis, stabilised the upper 3 cm of the sediment by compacting it, and Rowe (1974) stated that the shear strength of the sediment adjacent to the burrows of the anemone, Cerianthus, was almost twice as great as that of the sediment 20 cm away. Tubes and burrows of tanaids, polychaetes and harpacticoid copepods in subtidal algal mats can increase the stability of the sediment (Neumann et al., 1970; Pamatmat, 1968). Bock and Moore (1968) noted the stabilisation of sediment in the Gulf of Mexico by polychaete tubes.

Laboratory studies by Rhoads et al. (1978) showed that the fine mucous tubes of the capitellid polychaete, Heteromastus filiformis, increased the critical erosion velocity of the sediment thereby making it more resistant to erosion. The effect of bioturbation on the initiation of motion of intertidal sand has been studied in laboratory flume experiments (Grant et al., 1982).

(b) Effect of benthic invertebrates on sediment destabilisation

Burrowing and tube building of benthic invertebrates can destabilise sediments allowing them to be eroded easily by water currents. Dillon and Zimmerman (1970) found that burrowing by crabs caused erosion of submarine canyons and resulted in collapse of the canyon walls. Ott et al. (1976) estimated that burrowing and expulsion of sediment from the burrows of Upogebia littoralis caused the sediment surface to be eroded by up to 0.5 cm per year. The extensive burrowing by the mud crab, Panopeus herbsti, caused subsidence of the creek banks in which it lived (Edwards and Frey, 1977), and burrowing by the fiddler crab, Uca pugnax, caused considerable erosion of salt marsh sediment (Katz, 1980). Finally, the burrows of Panopeus herbsti, Sesarma reticulatum and Uca pugnax, which occupied 45% of the sediment surface area, decreased the shear strength of creek banks causing their subsequent collapse (Letzsch and Frey, 1980).

The intensive burrowing of subtidal muds by deposit-feeding organisms in Buzzards Bay and Cape Cod Bay, Massachusetts, produces a granular surface layer 5-10 mm thick (Rhoads, 1970). Such thixotropic muds are easily resuspended by weak tidal currents. Rhoads (1970) found that this uncompacted zone contains more than 60% water. While less intensively burrowed muds lack the granular surface texture, contain less than 50% water, and have plastic properties.

A high water content, an irregular surface and the low density of the constituent pellets destabilises the sediment surface and increases its erodibility. Tevesz et al. (1980) found that tubificid oligochaetes selectively ingest silt- to clay-sized particles at depths within the substratum, transporting them vertically through their gut and depositing them as faeces at the sediment-water interface. These activities form three distinct sedimentary layers. The sediment-water interface becomes covered with sand-sized faecal pellets. A slit-clay layer forms directly below this. The third layer is a sandy concentrate that represents the zone of feeding. The upper, pellitised layer has a high water content and is enriched in organic carbon, and is thus easily eroded.

Sediment destabilisation by the tube-building polychaete worm, Owenia fusiformis, has been studied in laboratory flume experiments (Eckman et al., 1981). These authors found that the bed was destabilised at all densities of tubes tested and this destabilisation was more pronounced at the higher densities.

(c) Effect of benthic invertebrates on sediment chemistry at the sediment-water interface

The way in which burrows and tubes influence the chemistry of marine sediments, and the exchange of organic ions across the sediment-water interface has been studied extensively recently (Aller, 1978a,b, 1980, 1982, 1983; Aller and Yingst, 1978; Berner, 1980; Day, 1978; Gust and Harrison, 1981; Kikuchi and Kurihara, 1977; McCaffrey et al., 1980; Mortimer, 1941; Nichols, 1974; Waslenchuk et al., 1983).

Aller (1978a) studied the effects of animal-sediment interactions on geochemical processes near the sediment-water interface. He found that in contrast to factors which cause heterogeneity, mobile infauna rapidly homogenise sediment and presumably simplify internal gradients.

From his study, he concluded that in bottom areas inhabited by long-lived sedentary infauna, measurable horizontal as well as vertical gradients in sediment chemistry may develop. However, in regions inhabited predominantly by mobile benthos, burrow positions change rapidly and horizontal gradients will be greatly reduced.

The diffusive permeability of animal burrow linings is important in determining marine sediment chemistry (Aller, 1983). Many of the abundant burrows formed by animals in sediments are lined with organic material. The permeability of these linings to solute diffusion can be an important determinant of the chemical composition of surrounding sediment and the burrow habitat.

Kikuchi and Kurihara (1977) have studied the effects of tubificid worms on submerged rice-field soil and overlying water. The worms destroyed the oxidised superficial soil layer by mixing the soil, altering the size composition of soil particles by their selective feeding on the small size fraction, and allowing a free exchange of dissolved substances between soil and the overlying water. As the oxidised surface soil layer was destroyed, iron and ammonia appeared in high concentrations in the water and sulphate-reducing bacteria were released.

(d) Effect of benthic invertebrates on sedimentation

Lynch and Harrison (1970) studied sedimentation caused by a tube-building amphipod, Ampelisca abdita, over a period of four months in the York River, Virginia, U.S.A. They noticed that numerous colonies of amphipods projected their tubes several centimetres above the river bottom. The tops of the amphipod tubes acted as sediment traps, building up the surface of the colony at a faster rate than the surrounding bottom.

The effect of deposit feeding oligochaetes on particle size and settling velocity of Lake Erie sediments has been studied by McCall (1979). He found that sediment collected from the western basin of Lake Erie has a median particle size of $1.5 \mu\text{m}$ and a median settling velocity of $0.0002 \text{ cm sec}^{-1}$. Microscopic examination showed that most of the particles were bound into layer cylindrical aggregates by the feeding activities of the oligochaetes (average pellet size = $280 \mu\text{m}$ length x $70 \mu\text{m}$ diameter). His laboratory experiments and field observations indicate that for most of the year the top 0.5-1 cm of the sediment is pelletised.

The effects of biological activity on the entrainment of marine sediment has been studied in laboratory experiments by Nowell et al. (1981). Effects of three species of polychaetes and two species of bivalves were tested in a flume. Tracks produced by animals at the sediment/water interface doubled the boundary roughness and decreased the critical entrainment velocity by 20%. Faecal mound sediment, which is bound together by mucus was less easily entrained than the surrounding sediment.

Pellet production accelerates the downward transfer of suspended detritus, resulting in deposition of much of the fine sediment that would otherwise escape the lake in interflows. Smith and Syvitski (1982) studied the role of pelletisation on deposition of fine-grained suspensates. They found that the mechanisms apparently responsible for bringing fine suspensates quickly to the bottom was pelletisation by sediment-ingesting zooplankton. The pellets, probably produced by pelagic copepods, are ovoidal with lengths typically ranging between 100 and $250 \mu\text{m}$ and are composed of clay and silt.

(IV) Secretory cells in the skin of annelids

Since four out of five of the species used in the experiments in the present section belong to the phylum Annelida, I will briefly describe the histology of the skin of this phylum.

1. Skin layers

The annelid epidermis is overlaid by a collagenous cuticle and rests on a basement membrane consisting of a thin, amorphous basal lamina subtended by collagen fibres in most large forms (Richards, 1978).

2. Gland cells

Gland cells, producing a range of muco-substances, are prominent in the polychaete and oligochaete epidermis (Richards, 1978). Three types of gland cells have been identified.

- (i) Large granular, orthochromatic cells secrete a mucopolysaccharide protein-lipid complex which functions as a viscoelastic lubricant in locomotion (Richards, 1973).
- (ii) Reticulate metachromatic cells produce a carboxylated acid mucus lacking uronic and sialic groups (Richards, 1974a).
- (iii) The sparsely occurring small granular cells which are protein-rich (Richards, 1974b) produces secretions that may affect the viscosity of the other epidermal secretions. These three types of gland cell are shown in Figure 37.

The presence of two types of mucous cells, one acid and one neutral, is common in polychaetes (Baffoni, 1968; Daly, 1973; Kryvi, 1971) and an additional proteinaceous type occurs in Nereis (Dorsett and Hyde, 1970).

(V) Secretory glands in the epidermis of Crustacea

Arthropod exoskeleton consists of a chitin-protein structure secreted over the body surface by an epidermis of a single layer of cells (Dennell, 1960). The chitin is of the α -type. The cuticle is usually hardened over most of the body by the deposition of calcium salts or by tanning by interaction between protein and quinone.

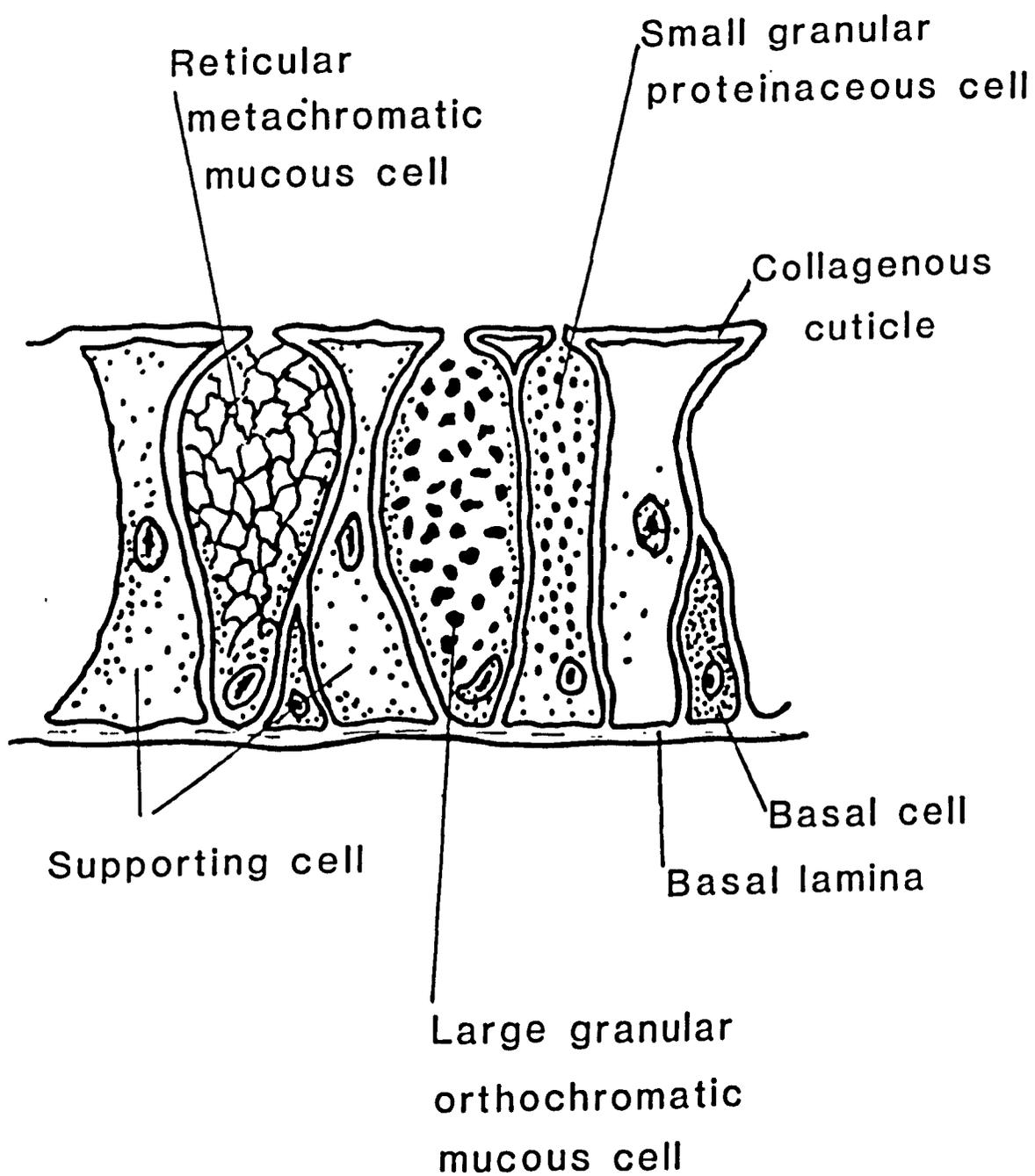
The structure of the crustacean cuticle is best known in the decapods. Four main strata are recognisable (Dennell, 1960; Ettershank et al., 1972; Lockwood, 1968). They are the epicuticle, the pigmented layer, the calcified layer and the uncalcified layer (Figure 38).

Little is known about mucus secretions in Crustacea, although the tegumental glands are thought to be involved. The tegumental glands are situated beneath the epidermis, and their ducts pass through the cuticle. They have been described in the Crustacea by many workers (Gorvett, 1946, 1951, 1952; Yonge, 1932). Tegumental glands are associated with cuticle formation (Gorvett, 1946; Yonge, 1932). In the decapod Crustacea, some of these glands may also produce mucus (Yonge, 1932).

Gorvett (1952) studied the tegumental glands in twelve species of land isopods concentrating on Porcellio scaber Latr. He studied the properties of secretions and the mode of action of the lobed glands (i.e. uropod and lateral plate glands) which are specialised tegumental glands unique to the Oniscoidea. Secretions from both glands solidify

Figure 37

Lumbricid earthworm epidermis with three types of secretory cell, supporting and basal cells (modified from Richards, 1978, p. 42).

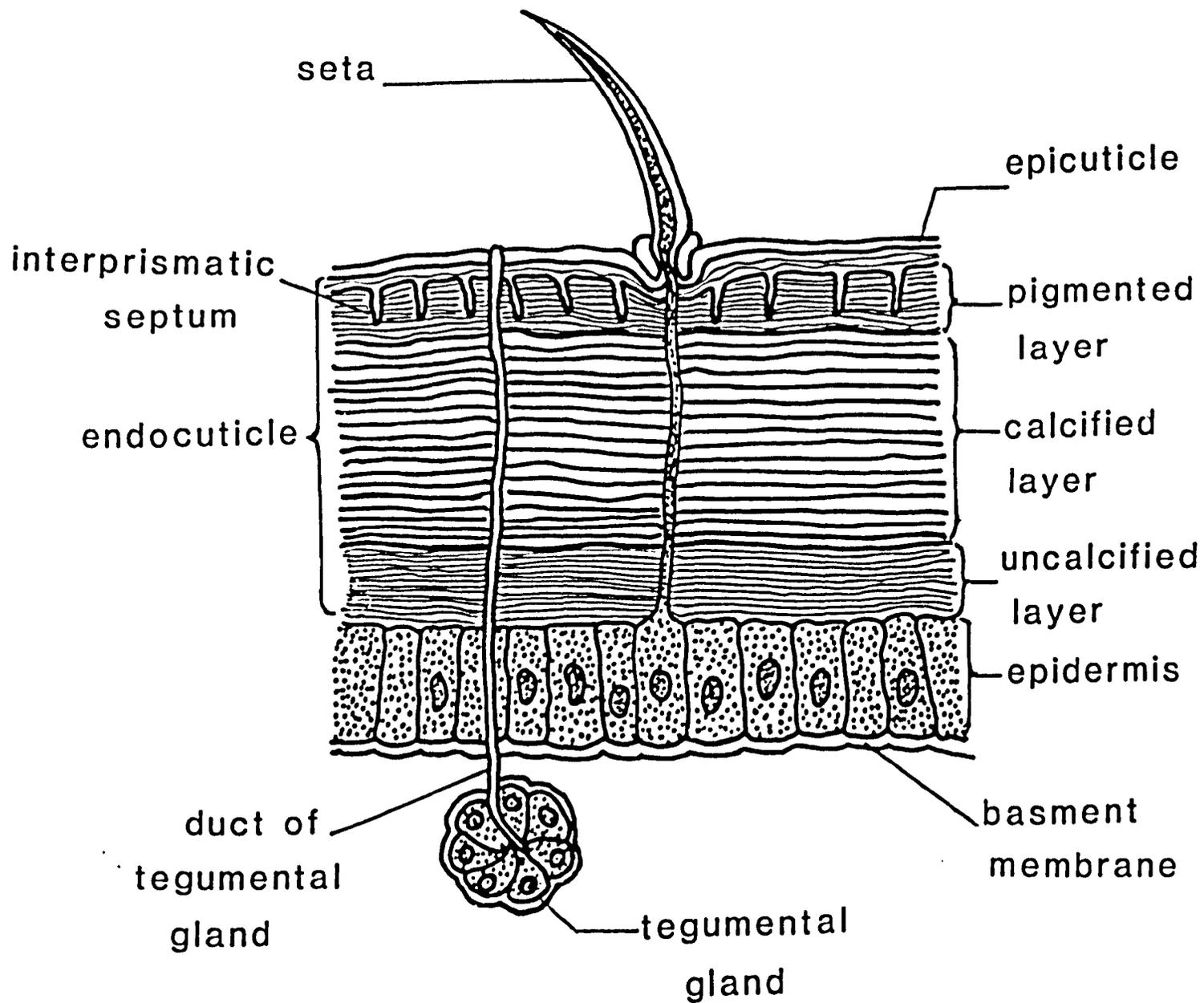


rapidly on exposure to air, and are coagulated by alcohol. The secretions form a hard translucent solid within a few seconds in the absence of oxygen, apparently by loss of water. The fresh secretion from both types of glands is a clear fluid which is soluble in water, and contains protein and possibly some fatty acid. It contains no trace of mucin, fat, wax, or glycogen. Gorvett (1956) quotes evidence from various sources suggesting that the function of the secretions is as a defence against spiders.

Some amphipods including Corophium volutator are known to produce secretions in tube or nest building. Besides Corophium volutator, Corophium bonellii and Corophium lacustre also build tubes. Schäfer (1972) records Cerapus crassicornis as living in membranous tubes at the surface of the sediment, Siphonoecetes species as living in mucus tubes covered with sand grains, and Erichthonius species as building mud tubes on hydroid polyps (pp. 311, 312). Amphitoe rubricata builds a nest with mucus-cemented walls among sea weed (Skutch, 1926). Bate and Westwood (1862) record the nests of this species as constructed of bits of weed, chiefly green Ulva, matted together with very delicate fibres which have the appearance of being spun or twisted. These authors also record Parajassa pelagica (Podocerus capillatus in Bate and Westwood, 1862), producing nests consisting of fine thread-like materials woven and interlaced, being established firmly in the branches of zoophytes. Various Siphonoecetes species are also recorded by Bate and Westwood (1862) as building tubes, although the taxonomy of this genus was very confused until the appearance of Myers and Mcgrath's paper (1979) (see Lincoln, 1979, p. 540) and so it is not possible to say which species Bate and Westwood were describing. The nature of the secretory glands of tube-building gammarids has been described by Nebeski (1880).

Figure 38

Cancer pagurus (Arthropoda - Crustacea) cuticle and
epidermis (modified from Dennell, 1960 and
Lockwood, 1968).



(VI) The mucous-lined burrow and mucus secretions

The construction of mucous burrows by benthic animals has been described in general by Schäfer (1972). The simplest way to transform a digging trail through the sediment into an open burrow with self-supporting walls, is to coat the sediment particles with mucus. This mucus is secreted by cells that are either scattered over the body surface (i.e. Annelida group) or are concentrated in certain places (i.e. Corophium). Animals with scattered mucous glands simply coat the burrow walls while the body glides back and forth in the sediment. The mucus penetrates into the spaces between sediment particles and cements them as soon as it hardens. While the sediment layer is soaked with secretion but is still yielding, the animal smooths and adjusts the inner space to fit the cross-section of the body. Finally, additional films of secretion cover the internal walls of the burrow in a more or less thick coating and smooth them perfectly. Where the secreting cells are limited to certain regions of the body, the construction becomes a concerted action performed by a specialised structure or appendage.

In the Amphipoda, tube construction is peculiar to the Ampeliscidae and Corophioidea, and a variety of materials, such as mud, clay, sand grains and shell and plant fragments may be used (Barnes, 1980). These materials are usually bound together with the aid of cementing secretions produced from glands of the second pereopods. These materials with their binding secretions keep the walls of the burrow in tact (Schäfer, 1972).

Extensive studies have been made on the participation of mucus secretions in tube-building polychaetes, and the earlier histochemical and biochemical information has been reviewed by Defretin (1971). Acid-mucins feature prominently in tube secretions, and hyaluronic acid is

reported in species secreting viscous tubes. The various mucosubstances may form the major part of the tube, or act as the matrix for sand grains, shell fragments or, in the case of serpulids, for calcium deposits. Tubes are often of a double nature, the outer part possessing the incorporated material and the inner lining being of mucus; in the newly-settled stages of Pectinaria an organic primordial tube precedes the formation of the double tube (Vovelle, 1973).

Table 47 summarises references on the chemical constituents of the secretions in polychaetes and other benthic organisms.

Inspection of the references in the above table shows:

1. fifty-four per cent of the cases were polysaccharides;
2. thirty-one per cent of the cases were mucoprotein;
3. eight per cent of the cases were organic sheets;
4. the remaining 7% were hyaluronic acid (5%), and in one case, there was no reaction with any of the reagents used.

(VII) The selected enzymes used

The enzymes I used were: α -amylase, hyaluronidase, lipase, lysozyme, pepsin and trypsin.

These enzymes were selected to enable me to ascertain something of the biochemical nature of the secretions I studied.

1. α -amylase

Enzymes catalysing the hydrolysis of: α - 1 — 4 glucosidic linkage of polysaccharides such as starch, glycogen, or their degradation products (Windloz, 1976). There are two types of α -amylase, from animal and from plant sources. Reaction catalysed:

TABLE 47. Representative references on secretions of benthic invertebrates and some vertebrate animals

| Author | Year | Studied group | Compound identified "chemical structure and type" of materials |
|---------------------------|------|---|---|
| Aller and Yingst | 1978 | Annelida - Polychaeta - sedentary | Organic sheets |
| Chandler and Fleeger | 1984 | Crustacea - Copepoda | Mucopolysaccharides |
| Daly | 1973 | Annelida - Polychaeta - Nereidae | Acid-mucopolysaccharides |
| Defretin | 1971 | A wide range of Annelida - Polychaeta | Acid-mucopolysaccharides; hyaluronic acid; mucopolysaccharides; horny materials resemble chitin |
| Ewer and Hanson | 1945 | A wide range of Annelida - Polychaeta A range of Mollusca species One species of coelenterates One species of phylum Cnidaria One species of phylum Nemertina | Mucoprotein " " " |
| Fager | 1964 | One species of Annelida - Polychaeta (<u>Owenia fusiform</u>) | Organic layer, of a quick-settling proteinaceous materials |
| Hunt | 1970 | One species of Annelida - Polychaeta (<u>Hyalinoecia tubicola</u>) | Polysaccharide phosphate onuphic acid |
| Rahemtulla and Løvtrup | 1974 | Annelida (<u>Tubifex</u>) | Mucopolysaccharides |

Continued overleaf

TABLE 47 (Continued)

| Author | Year | Studied group | Compound identified "chemical structure and type" of materials |
|------------------------|------|--|--|
| Risk and Szcuczek | 1977 | Trace fossils - burrows of crustacean worm tubes; feeding burrows of deposit-feeder; Mollusca - bivalve and other fossils | Polysaccharides |
| Shillaker and Moore | 1978 | Crustacea - Amphipoda | Acid and sulphated mucopolysaccharides |
| Zola | 1967 | Annelida - Polychaeta (<u>Chaetopterus variopedatus</u>) | Phosphorous rich polysaccharides |
| Vovelle | 1973 | Annelid - Polychaeta (<u>Pectinaria</u>) | Organic |

Starch or glycogen \longrightarrow dextrans + maltose

2. Hyaluronidase

The enzyme hyaluronidase catalyses hydrolysis of the $\beta(1 \rightarrow 4)$ linkages of hyaluronic acid; this hydrolysis is accompanied by a decrease in viscosity (Lehninger, 1975, p. 273).

3. Lipase

An enzyme (or more exactly a group of enzymes) belonging to the esterases. Hydrolyses fat (present in ester form, such as glycerides) yielding fatty acids and glycerol (Windloz, 1976).

4. Lysozyme

Lysozyme dissolves certain bacteria by cleaving the polysaccharide component of their cell wall (Stryer, 1975).

The cell wall polysaccharide is made up of two kinds of sugars: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) (Stryer, 1975). NAM and NAG are derivatives of glucosamine in which the amino group is acetylated. In bacterial cell walls, NAM and NAG are joined by glycosidic linkages between C-1 of one sugar and C-4 of the other. Lysozyme hydrolyses the glycosidic bond between C-1 of NAM and C-4 of NAG (see Stryer, 1975, p. 136, for details).

5. Pepsin

Principal digestive enzyme of gastric juice, controls the degradation of proteins to proteoses, peptones and amino acids (Stryer, 1975; Windloz, 1976).

6. Trypsin

Trypsin splits peptide bonds on the carboxyl side of lysine and arginine residues only. It also hydrolyses esters and amides containing this bond (Stryer, 1975, p. 104).

(VIII) Aims of the present section

The aims of the present section are:

1. to assess the effects of benthic invertebrate secretions on sedimentation;
2. to obtain some idea of the chemical nature of the secretions by enzymic digestion;
3. to test how enzymic digestion of secretions alters the effect of these secretions on sedimentation;
4. to refine the sedimentation procedure following some apparently anomalous results.

SECTION III

Materials and Methods

Three types of sediments were used in these experiments: Langbank, Ardmore and Rockware (see Section I, p. 32). The Materials and Methods are divided into three parts. The first gives the effect of animal secretions on sedimentation; the second gives the effect of different enzymes on animal secretions; the third describes how enzymic digestion alters the effect that secretions have on sedimentation.

(I) Effect of animal secretions on sedimentation

Samples from Langbank and Ardmore sediments were transported to the aquarium, carefully sieved and maintained in aerated sea water (see Section II, p. 184). Sediments from the sampling sites contained different species (see Section I, p. 32). Corophium volutator and Nereis diversicolor were used from Langbank, and Pygospio elegans, Scoloplos armiger and Arenicola marina from Ardmore. The species were separated from sediments and kept in separate dishes.

The secretions of Langbank species were tested against Langbank and Rockware sediments and the secretions of Ardmore species were tested against Ardmore and Rockware sediments. The overall plan of these experiments is shown in Table 48.

1. Effect of Langbank species on sedimentation (experimental series 1 and 2)

Langbank or Rockware sediment was placed in one litre beakers of 10 cm diameter. The beakers were filled with sediment to a height of 2 cm below their tops. Separate beakers were prepared for each species (i.e. C. volutator and N. diversicolor). Another beaker was prepared containing both C. volutator and N. diversicolor to study the effect of mixed secretions. Control beakers containing sieved sediment with no

animals were also prepared.

The numbers of animals in each beaker was calculated as follows. The natural population density at mid-tide level at Langbank for C. volutator and N. diversicolor is:

7980/m² (equivalent to 0.7980/cm²) for C. volutator
and 1380/m² (equivalent to 0.1380/cm²) for N. diversicolor.

The area (A) of the top of the beaker was calculated as:

$$A = r^2 \times \frac{22}{7} \quad (r = 5 \text{ cm}).$$

The approximate number of animals/beaker was calculated as:

0.7980 x (25 x $\frac{22}{7}$) \approx 63 animals/beaker for C. volutator,
and 0.1380 x (25 x $\frac{22}{7}$) \approx 11 animals/beaker for N. diversicolor.

Animal density in each experimental dish was set at 70 for C. volutator and at 15 for N. diversicolor. The mixed beaker contained 70 C. volutator and 15 N. diversicolor.

2. Effect of Ardmore species on sedimentation (experimental series 3 and 4)

Ardmore or Rockware sediment was placed in crystallising dishes. The dishes were filled with the sediment to a height of 2 cm below their tops. Separate dishes were prepared for each species (i.e. P. elegans, S. armiger and A. marina). Two different sizes of dishes were used - large for A. marina and small for P. elegans and S. armiger. No mixed species dishes were used in this experimental series. Control dishes containing sieved sediment with no animals were also maintained.

The number of animals in each dish was calculated as follows. The natural population density at mid-tide level at Ardmore for P. elegans, S. armiger and A. marina is:

50-100 animals/core (equivalent to $16968.3/m^2 = 1.70/cm^2$)

for P. elegans (by taking the mean of 50-100/core = 75);

$180/m^2$ (equivalent to 2/small dish) for S. armiger;

and $30/m^2$ (equivalent to 2/large dish) for A. marina.

The area (A) of the top of the small and large dishes were calculated as:

$$A = r^2 \times \frac{22}{7} \quad (r = 3.75 \text{ cm for the small dish,} \\ r = 9.25 \text{ cm for the large dish)}$$

The approximate number of animals/dish was calculated as

$$1.7 \times (3.75^2 \times \frac{22}{7}) \approx 75 \text{ animals/small dish for } \underline{P. elegans};$$

$$0.018 \times (3.75^2 \times \frac{22}{7}) \approx 1 \text{ animal/small dish for } \underline{S. armiger}$$

$$\text{and } 0.003 \times (9.25^2 \times \frac{22}{7}) \approx 1 \text{ animal/large dish for } \underline{A. marina}.$$

Animal density in each experimental dish was set at 75 P. elegans and at 2 for each of S. armiger and A. marina.

TABLE 48. Plan of experiments showing species and sediments used. In each experimental series, the sedimentation of the four treatments (i) to (iv) was tested at the same time so that accurate comparisons could be made. Approximately six one litre beakers were used for each combination (✓).

| Treatments | Experimental series and sediments used | |
|---|--|-------------------------------|
| | Series 1 Langbank sediment | Series 2 Rockware sediment |
| <u>Langbank species</u> | | |
| (i) Control (no species) | ✓ | ✓ |
| (ii) <u>C. volutator</u> | ✓ | ✓ |
| (iii) <u>N. diversicolor</u> | ✓ | ✓ |
| (iv) <u>C. volutator</u> plus <u>N. diversicolor</u> | ✓ | ✓ |
| <u>Ardmore species</u> | Series 3 Ardmore sediment | Series 4 Rockware sediment |
| (i) Control (no species) | ✓ | ✓ |
| (ii) <u>P. elegans</u> | ✓ | ✓ |
| (iii) <u>S. armiger</u> | ✓ | ✓ |
| (iv) <u>A. marina</u> | ✓ | ✓ |

3. Treatment of dishes

After the species had been placed in the experimental dishes, the dishes were covered with a mesh cloth which was secured with an elastic band - thus preventing the escape of animals. The dishes were then maintained for seven days in an aquarium at 10°C in large plastic containers under running sea water.

After seven days the upper 1.5 cm of sediment was removed from each dish and spread on a metal foil sheet. All animals were carefully removed using fine forceps. The sediment was then mixed gently using a spatula. Fifty-five grammes of the mixed sediment was taken and used for sedimentation (see Section II, p. 200).

Secretions were also prepared for scanning electron microscopy. The preservation procedure and a summary of the sample treatments are shown below. However, the details of the scanning electron microscopy methodology are present in Appendix III, pp. 553 to 559.

4. Preservation of samples

Secretions with attached sand grains were fixed, stained and stored (Meadows and Anderson, 1968; see Section II, p. 201 for details).

Scanning electron microscopy was carried out on the samples as follows.

(a) Dehydration

Samples were dehydrated through an acetone series: 30%, 50%, 70%, 90% and anhydrous acetone. Samples were washed twice with each concentration of acetone (5 min/wash).

(b) Critical point drying

The dehydrated samples were transferred to separate metal baskets ensuring that the samples were kept under acetone to prevent rehydration. Samples were then quickly placed in the critical point drying apparatus. Critical point drying was conducted over one hour.

(c) Gold coating

Stubs of the critical point dried samples were prepared and placed in a sputter coater apparatus for 30 minutes to coat them with a thin layer of gold ($\approx 50 \text{ nm} = 500 \text{ \AA}$).

(d) At this stage, the samples were ready to be photographed with the scanning electron microscope (details for all the above steps are present in Appendix III, pp. 553 to 559).

(II) Effect of enzymes on animal secretions1. Preparation of buffers

Two types of buffers were prepared (Cruickshank, 1975).

(a) Citrate-phosphate bufferStock solution (i)

0.1 M solution of citric acid

$(\text{HO.CO.CH}_2.\text{C}(\text{OH})(\text{COOH}).\text{CH}_2\text{COOH.H}_2\text{O} = 210.14)$

(19.21 grammes in 1000 ml).

Stock solution (ii)

0.2 M solution of di-sodium hydrogen orthophosphate

anhydrous ($\text{Na}_2 \text{HPO}_4 = 141.96$).

Appropriate buffers were prepared by combining known volumes from the above stock solutions. X ml of stock solution (i) + y ml of stock solution (ii), diluted to a total of 100 ml.

| X | Y | pH |
|------|------|-----|
| 44.6 | 5.4 | 2.6 |
| 30.7 | 19.3 | 4.0 |

(b) Phosphate buffersStock solution (i)

0.2 M solution of monobasic sodium phosphate

(31.2 g NaH_2PO_4 in 1000 ml)

Stock solution (ii)

0.2 M solution of di-sodium hydrogen orthophosphate

anhydrous ($\text{Na}_2\text{HPO}_4 = 141.96$).

Appropriate buffers were prepared by combining known volumes from the above stock solutions. X ml of stock solution (i) + Y ml of stock solution (ii), diluted to a total of 200 ml.

| X | Y | pH |
|------|------|-----|
| 81.5 | 18.5 | 6.2 |
| 39.0 | 61.0 | 7.0 |
| 13.0 | 87.0 | 7.6 |

2. Preparation of enzyme solutions

Six enzymes were used: α -amylase, hyaluronidase, lipase, lysozyme, pepsin and trypsin. These enzymes have different digestive activities/unit weight. I decided to use 2000 units of activity for each enzyme. The required weight for each enzyme to get the 2000 units of activity was then calculated as follows.

$$\frac{2000 \text{ units}}{\text{units of activity}} = \text{required weight of each enzyme}$$

Weights, temperatures and pH required for each enzyme are shown in Table 49. Each enzyme solution was then made up with the appropriate buffer.

TABLE 49. Enzyme concentrations, temperature and pH used in experiments.

| Enzyme | Commercial supplier | Code | Concentration (mg/ml) | Temperature (°C) | pH |
|-------------------|---------------------|--------|-----------------------|------------------|-----|
| α -amylase | Sigma | A-1278 | 27.40 | 20 | 7.0 |
| Hyaluronidase | Sigma | H-3506 | 4.000 | 37 | 4.0 |
| Lipase | Sigma | L-1754 | 4.256 | 37 | 7.6 |
| Lysozyme | Sigma | L-2879 | 0.0500 | 20 | 6.2 |
| Pepsin | Sigma | P-7000 | 3.810 | 37 | 2.6 |
| Trypsin | Sigma | T-8128 | 1.149 | 20 | 7.0 |

3. Animal secretions

Animal secretions were prepared as in part (I) Materials and Methods using only Rockware sediment. Secretions from different species were kept separately.

4. Enzymes/animal secretions experiment

One gramme of the secretion was placed in a vial. The secretion was washed with the appropriate buffer to remove salt. Two different washing procedures were used. The first contained three, and the second five washing steps with buffer, before the addition of enzymes (Figure 39). Two experiments were conducted to test any differences between the three-wash and five-wash procedure. These experiments showed that the five-wash procedure was the more effective in allowing enzymes to subsequently digest the secretions (Figure 40 and Appendix III, Table 12, pp. 314, 528). Since less secretory material remained after five prior washes than after three. The data were analysed by two matched t-tests - one for the C. volutator data and one for the N. diversicolor data (Appendix III, Table 12). Both tests were highly significant, hence proving that five prior washes were better than three.

Two point five millilitres of the enzyme solution was then added to each of the different secretions in a vial. Each vial was placed in the appropriate temperature required for the enzyme reaction. Vials were left undisturbed for two hours. Control vials were kept with the experimental vials.

At the end of the two hours, enzyme solution were poured off. The vial contents were then washed three times with the appropriate buffers to remove the enzymes.

Samples were dried at 60°C in an oven for 24 hours and then placed in a desiccator to cool for 30 minutes. The grains attached to the

secretions remaining after enzymic digestion were removed, and the unattached grains weighed. The grains attached to secretions were then weighed, ashed in a furnace at 600°C for six hours, and reweighed. The weight lost during ashing was equal to the dry weight of the secretions remaining after enzymic digestion. The weight of grains remaining represents the weight of the attached grains.

5. Calculation of % weight of secretions remaining after enzymic digestion

These were calculated as a percentage of the mean weights of the appropriate controls (undigested secretions in buffer).

$$\% = \frac{\text{weight remaining after digestion} \times 100}{\text{meant weight of secretions in controls}}$$

2. In the part (I) results of the present section, it was shown that Scoloplos armiger did not have a noticeable effect on sedimentation of Ardmore and Rockware sediments. Therefore, I decided to exclude this species from all further experiments with Ardmore sediment. P. elegans and A. marina are the only two species which were included in the experiments.

Figure 39 .

Flow diagram of the two washing procedures used in
cleaning sediment particles containing animal secretions.

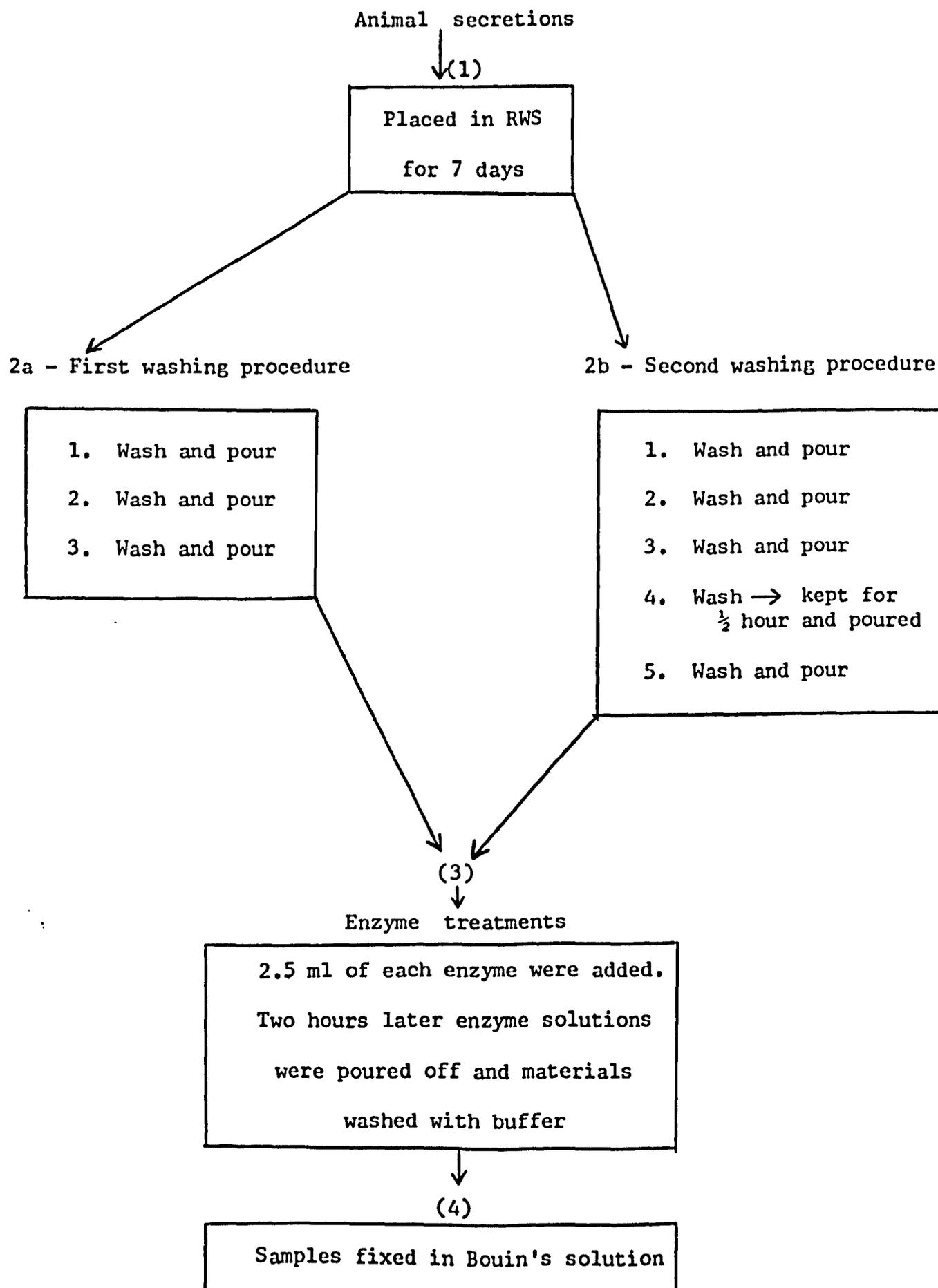
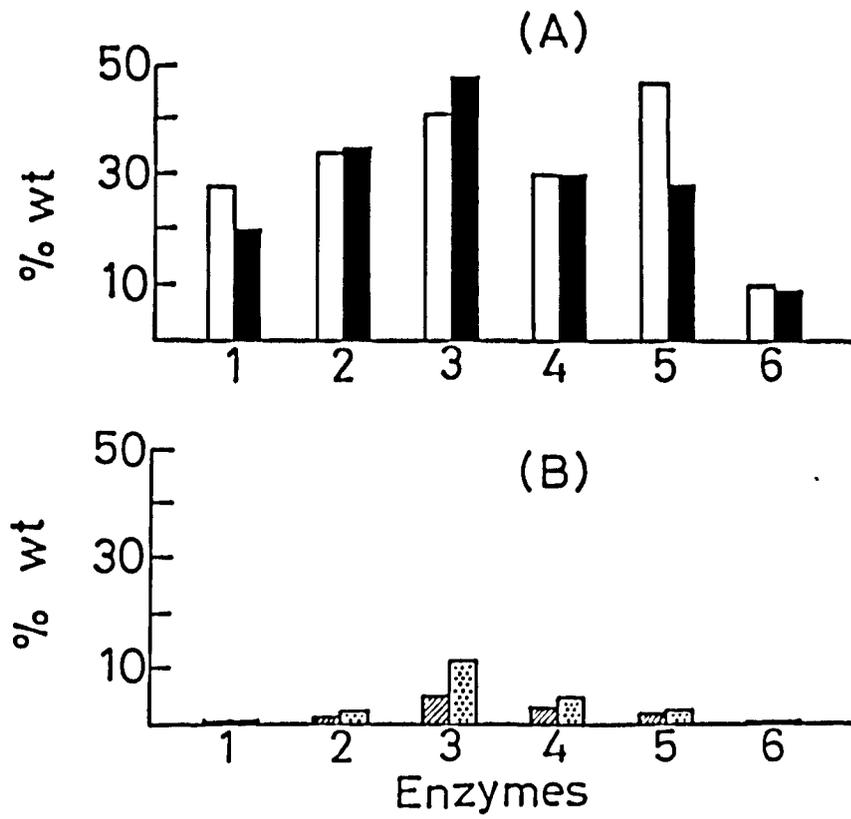
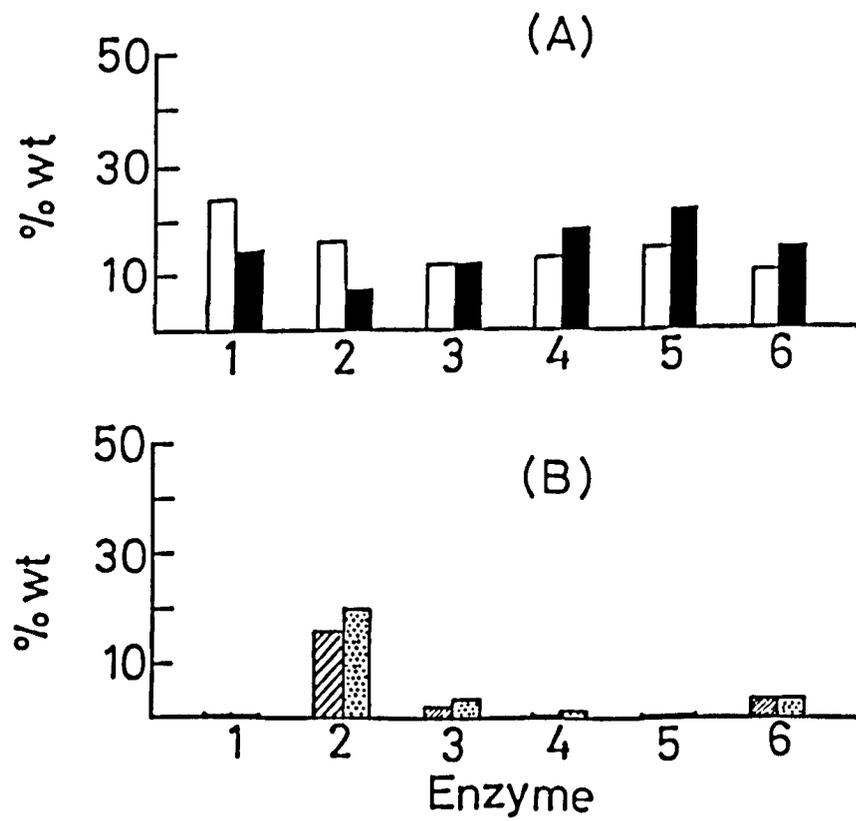


Figure 40

Effect of two prior washing procedures (A: three washes; B: five washes) on the subsequent enzymic digestion of secretions of C. volutator and N. diversicolor. The numbers on the x-axis refer to the different enzymes used: (1) α -amylase, (2) hyaluromidase, (3) lipase, (4) lysozyme, (5) pepsin, and (6) trypsin. On the y-axis, the weights of secretions remaining after enzymic digestion are expressed as a % weight of the controls. The different columns (\square , \blacksquare) and (\square , \square) represent replicates carried out for each washing procedure.

C. volutatorN. diversicolor

(III) Effect of enzymic digestion on secretions and sedimentation

From the previous experiments, it was clear that animal secretions have a profound effect on sedimentation (see Results section, part I, p. 320). It is also clear that the enzymes had different effects on the secretions (see Results section, part II, p. 355). The aim of the present section was to find out whether enzymic digestion of secretions affects sedimentation.

Animal secretions were prepared and treated with enzymes as previously (pp. 309). Because of the small volume of sediment used in the enzymic digestion experiments, it was necessary to scale down the sedimentation experiment. This was done by reducing the volume of sediment and sea water by reducing the number of replicates taken at each time interval from the sedimentation cylinder, and by preparing only one cylinder plus sediment for each sediment treatment - not six (i.e. one for each time).

Scaling down of the sedimentation experiment

Preliminary experiments were conducted in which the volume of synthetic sea water (500 ml) and the wet weight of sediment (55 g) was scaled down to 50 ml and 5 g respectively.

I used a stoppered 50 ml cylinder, and sampled at 5 cm rather than 10 cm below the water level. As previously this was done by marking the pipette on the outside. Two millilitre pipette samples were removed at 5, 10, 20, 40, 60 and 120 seconds. Two replicates were taken at each time interval. A 55 g/500 ml experiment was also conducted so that the two methods could be compared accurately. A number of preliminary trials of the 5 g/50 ml experiment were conducted beforehand to obtain expertise. The results of the definitive 5 g/50 ml experiment and the 55 g/500 ml experiment are shown in Table 50 and

Figure 41. The suspended weights (mg/ml) at 5 cm depth in the 5 g/50 ml experiment were very similar to those at 10 cm depth in the 55 g/500 ml experiment. This means that both experiments will give the same results despite the difference in sediment weight and sea water volume. As is to be expected, the standard deviations and the coefficients of variation were higher in the scaled experiment (Table 50).

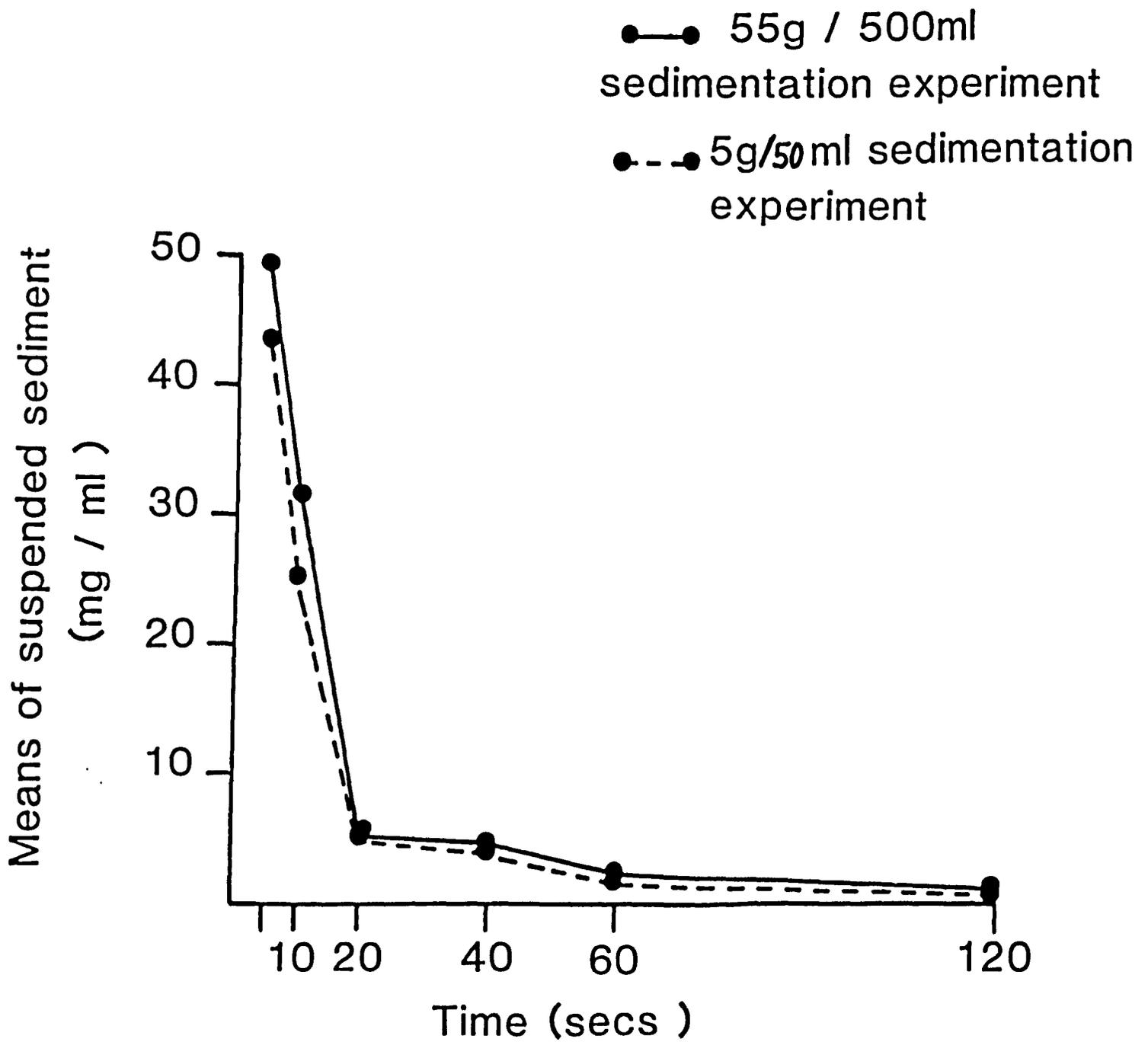
The following definitive procedure was therefore used in all the sedimentation experiments in which the effect of enzymes on secretions was tested. Fifty millilitres of sea water plus 5 g wet weight of sediment was placed in a 50 ml stoppered measuring cylinder. One cylinder plus sediment (rather than six) was prepared for each sediment treatment, and two (rather than five) replicates were removed at each time interval. The time intervals were 5, 10, 20, 40, 60 and 120 seconds. Each replicate removal consisted of a 2 ml sample taken with a 10 ml pipette at 5 cm depth below the water surface. The order in which the 6 x 2 replicates from a given cylinder were removed, was obtained from a table of random numbers (Rohlf and Sokal, 1969). The actual random sequences used for each cylinder are shown in Appendix III, Table 1 (p. 517).

TABLE 50. 55 g/500 ml and 5 g/50 ml sedimentation experiment. Means, standard deviations and coefficient of variance of suspended sediment (mg/ml) at 10 cm water depth (55 g/500 ml experiment) and at 5 cm water depth (5 g/50 ml experiment) (coefficient of variation = $\frac{s.d}{mean} \times 100\%$).

| Experiment | Factors | Time (secs) | | | | | |
|------------|---------|-------------|---------|---------|---------|---------|---------|
| | | 5 | 10 | 20 | 40 | 60 | 120 |
| 55g/500 ml | Mean | 49.83 | 31.78 | 5.275 | 4.925 | 2.150 | 1.020 |
| | s.d | 0.0559 | 0.07950 | 0.07289 | 0.03953 | 0.03953 | 0.03708 |
| | C. of V | 0.1122 | 0.2502 | 1.382 | 0.8036 | 1.839 | 3.635 |
| 5g/50. ml | Mean | 43.95 | 25.39 | 5.170 | 3.710 | 1.525 | 0.6500 |
| | s.d | 0.3690 | 0.3025 | 0.1006 | 0.1901 | 0.05863 | 0.2107 |
| | C. of V | 0.8396 | 1.191 | 1.946 | 5.124 | 3.845 | 32.42 |

Figure 41

55 g/500 ml (□) and 5 g/50 ml (■) sedimentation experiments. Means of suspended sediment dry weight (mg/ml) at 10 cm depth (55 g/500 ml experiment) and at 5 cm depth (5 g/50 ml experiment) below the water level. Standard deviations of the 55 g/500 ml experiment were so small that they could not be seen.



SECTION III

Results

The results of this section are divided into four parts. The first (I) gives the effect of animal secretions on sedimentation. The second (II) gives the effect of different enzymes on the animal secretions. The third (III) describes how enzymic digestion of secretions alters the effect that secretions have on sedimentation. The fourth (IV) presents a refinement of the sedimentation experiment; the materials, methods, and results of this part are presented separately at the end of the section.

(I) Effect of animal secretions on sedimentation

The results of this part are divided into four subdivisions: (1) the effect of Langbank species' secretions (Corophium volutator and Nereis diversicolor) on sedimentation; (2) the effect of Ardmore species' secretions (Pygospio elegans, Scoloplos armiger and Arenicola marina) on sedimentation; (3) statistical comparisons between the effect of Langbank species and Ardmore species on sedimentation of Rockware sediment; (4) scanning electron microscopy of the secretions.

1. Effect of Langbank species (C. volutator and N. diversicolor) on sedimentation of Langbank and Rockware sediments

The results of this part are shown in Figures 42 and 43 and Appendix III, Table 2, p. 518. Control sediment sediments very quickly, followed by sediment treated with C. volutator secretions, sediment treated with N. diversicolor secretions, and sediment treated with secretions of both species; there is little difference between the latter two treatments. These conclusions are substantiated by the following statistical analyses.

A series of 1 x 4 one-way analyses of variance were applied to the suspended weights (mg/ml) of the control sediment, the sediment containing C. volutator secretions, sediment containing N. diversicolor secretions and the sediment containing secretions of both species. These anovars were carried out for each of the six time intervals. The first six anovars were conducted on Langbank sediment containing Langbank species (Table 51). The second six anovars were conducted on Rockware sediment containing Langbank species (Table 52). The results from all the twelve one-way anovars were highly significant ($P < 0.001$).

The suspended weights (mg/ml) were then transformed to ln values (Appendix III, Table 3, p. 519) to obtain straight lines of the form $\ln y = \ln a + bx$, where $\ln y = \ln (\text{mg dry weight/ml})$ and x equals time (secs). These lines are shown in the lower half of Figures 42 and 43.

The straight line equations thus obtained allowed me to predict the time required for 50 and 95% sedimentation (Tables 53 and 54). As expected, animal secretions increased the 50% (x_{50}) and the 95% (x_{95}) time of both sediment types (cf. one-way anovars described above). An additional interesting conclusion can be made from the data. The x_{50} and x_{95} times are approximately the same for the control Langbank and Rockware sediments. However, although the secretions increased these times in both sediments, the increase was greater by a factor of c. 1.5 to 2.0 in Langbank sediment compared to Rockware sediment. For example, the x_{50} and x_{95} times with C. volutator secretions were 38.81 seconds and 167.7 seconds with the Rockware sediment and 59.39 seconds and 256.7 seconds with the Langbank sediment. The explanation for this difference is not clear but may reflect small differences in particle size between the two sediments or the production of more mucus in the natural sediment than in the artificial Rockware sediment.

Figure 42

Relationships between time intervals (secs) and suspended weights (mg dry weight/ml) of Langbank sediment. Weights obtained from the untreated (control) sediment and the treated sediment with animal secretions, C. volutator (■—■), N. diversicolor (O—O), and both species together (▲—▲). The upper plot shows the original suspended weights, while the lower plot shows the transformed data (ln of suspended weight). Each point is the mean of five replicate samples. Standard deviations are not shown because they are too small to plot. The means and standard deviations of the original data are given in Appendix III, Table 2, p. 518.

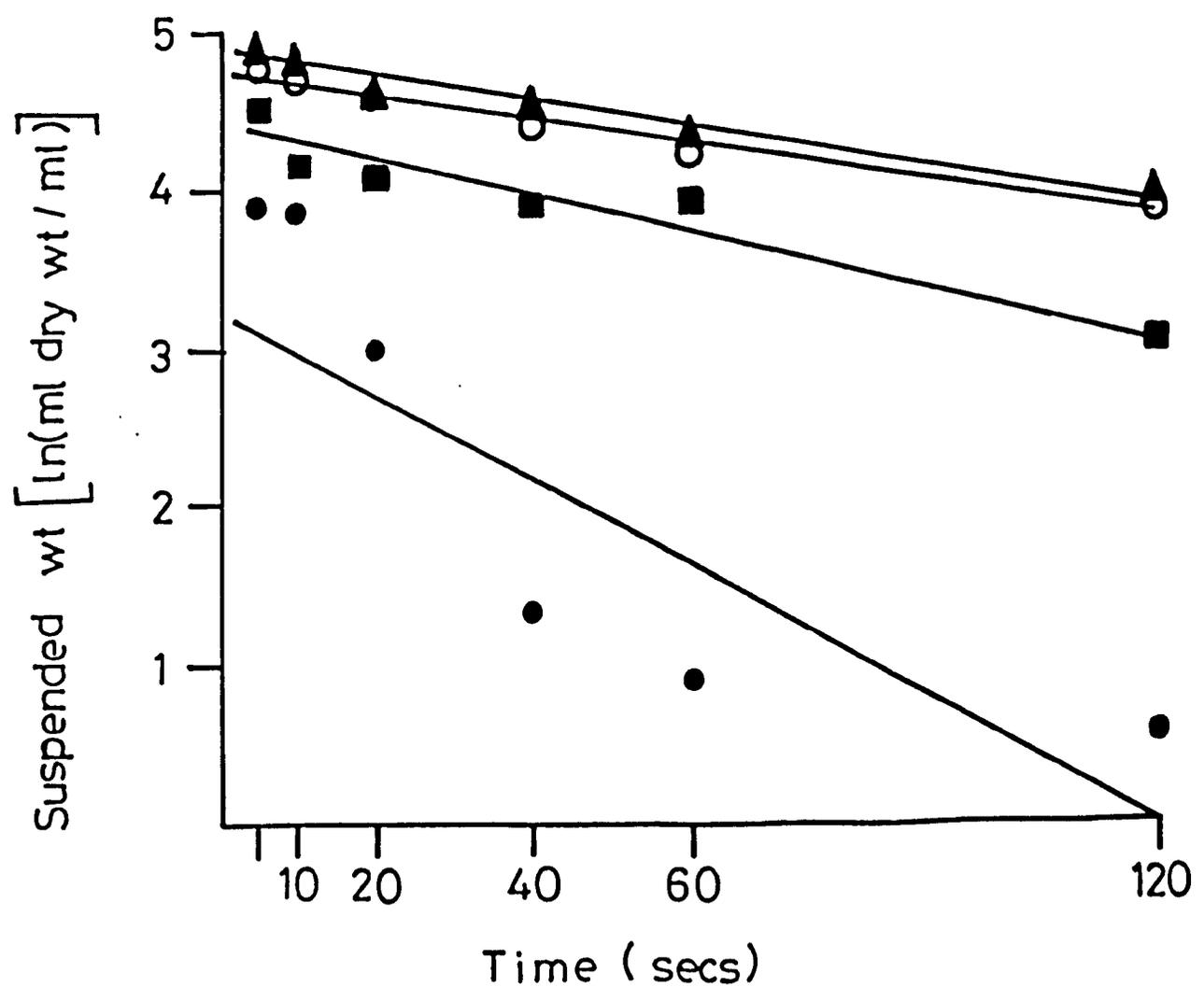
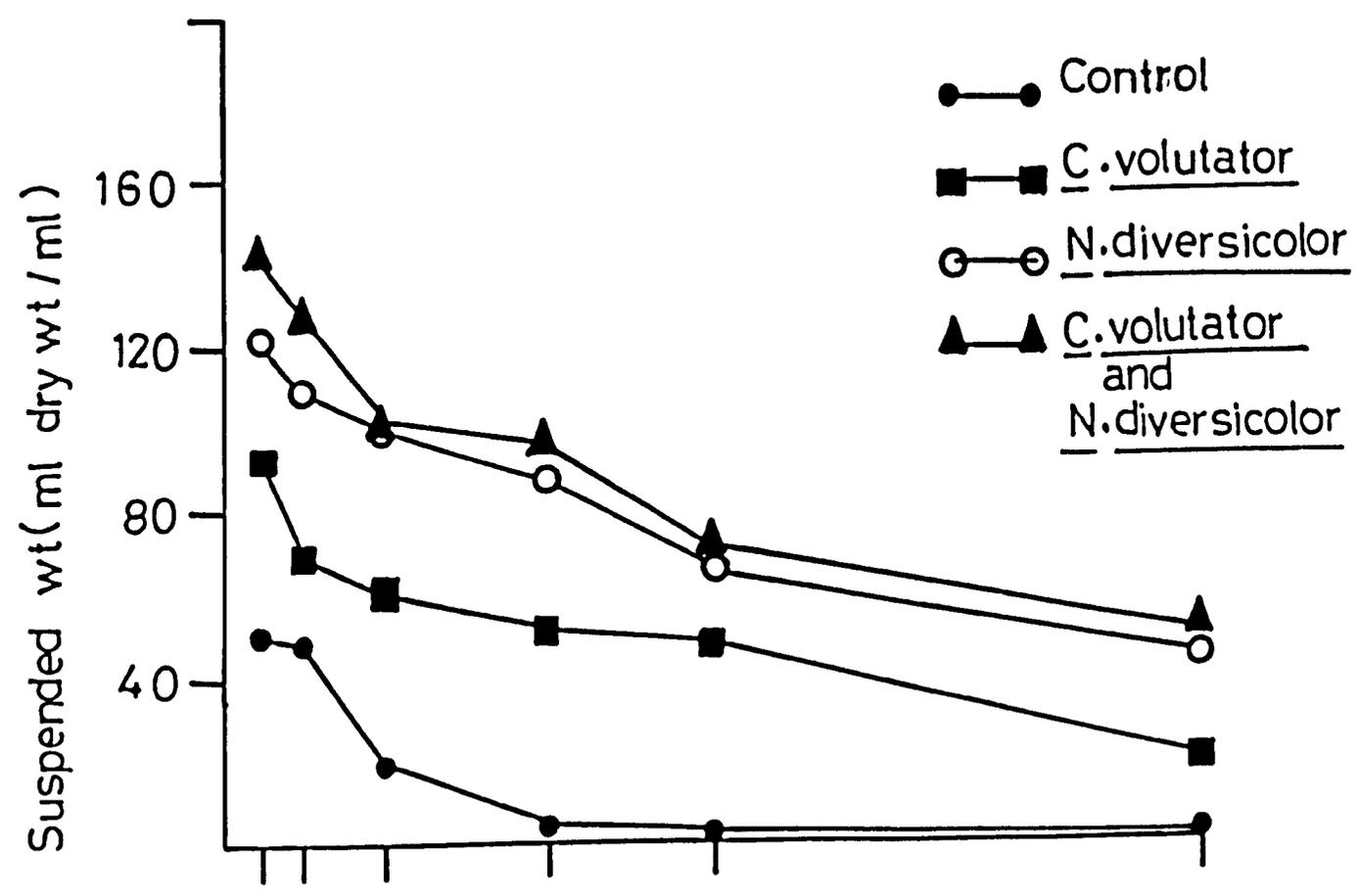


Figure 43

Relationships between time intervals (secs) and suspended weights (mg dry weight/ml) of Rockware sediment. Weights obtained from the untreated (control) sediment and the treated sediment with animal secretions, C. volutator (■—■), N. diversicolor (○—○), and both species together (▲—▲). The upper plot shows the original suspended weights, while the lower plot shows the transformed data (ln of suspended weight). Each point is the mean of five replicate samples. Standard deviations are not shown because they are too small to plot. The means and standard deviations of the original data are given in Appendix III, Table 2, p. 518.

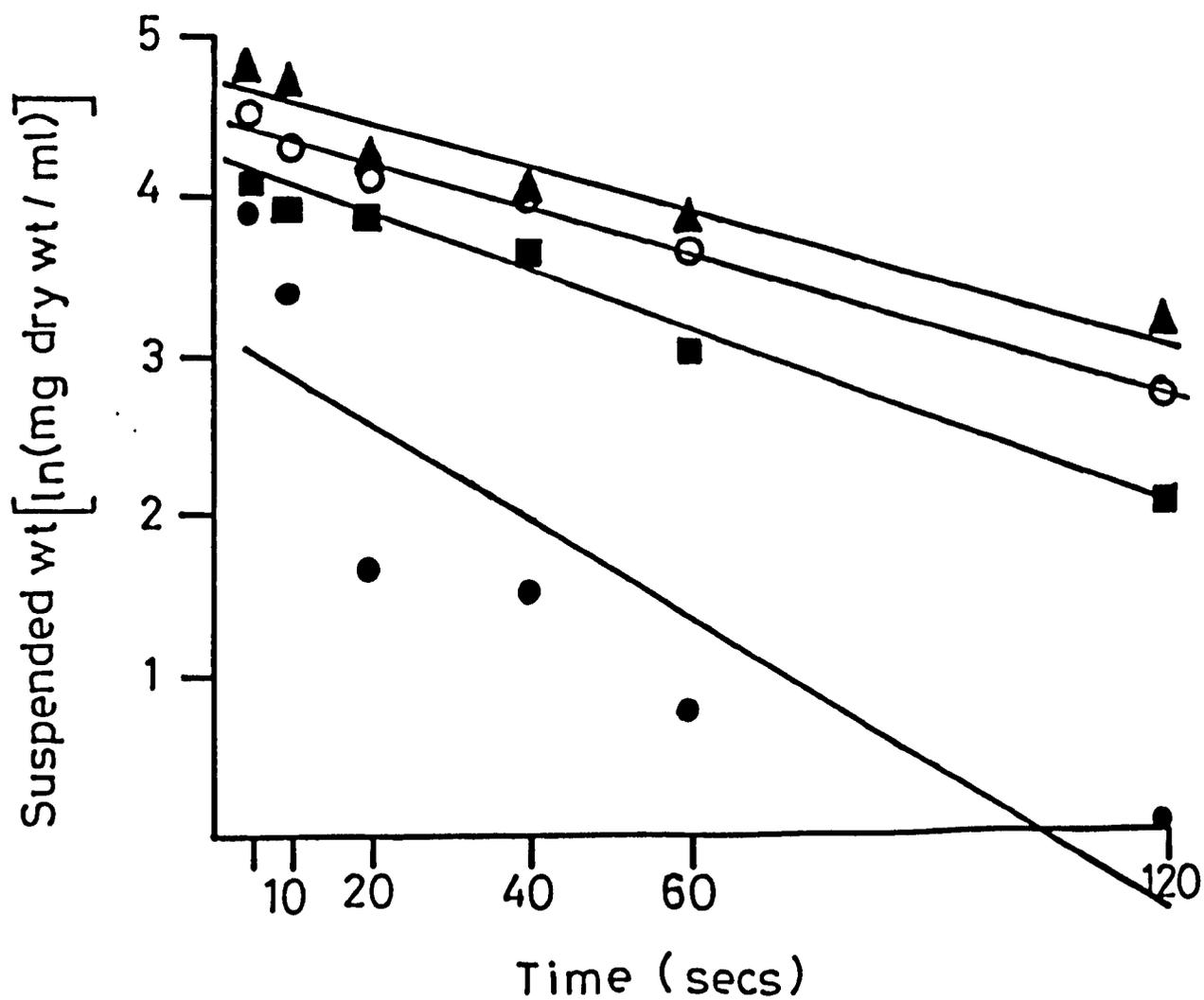
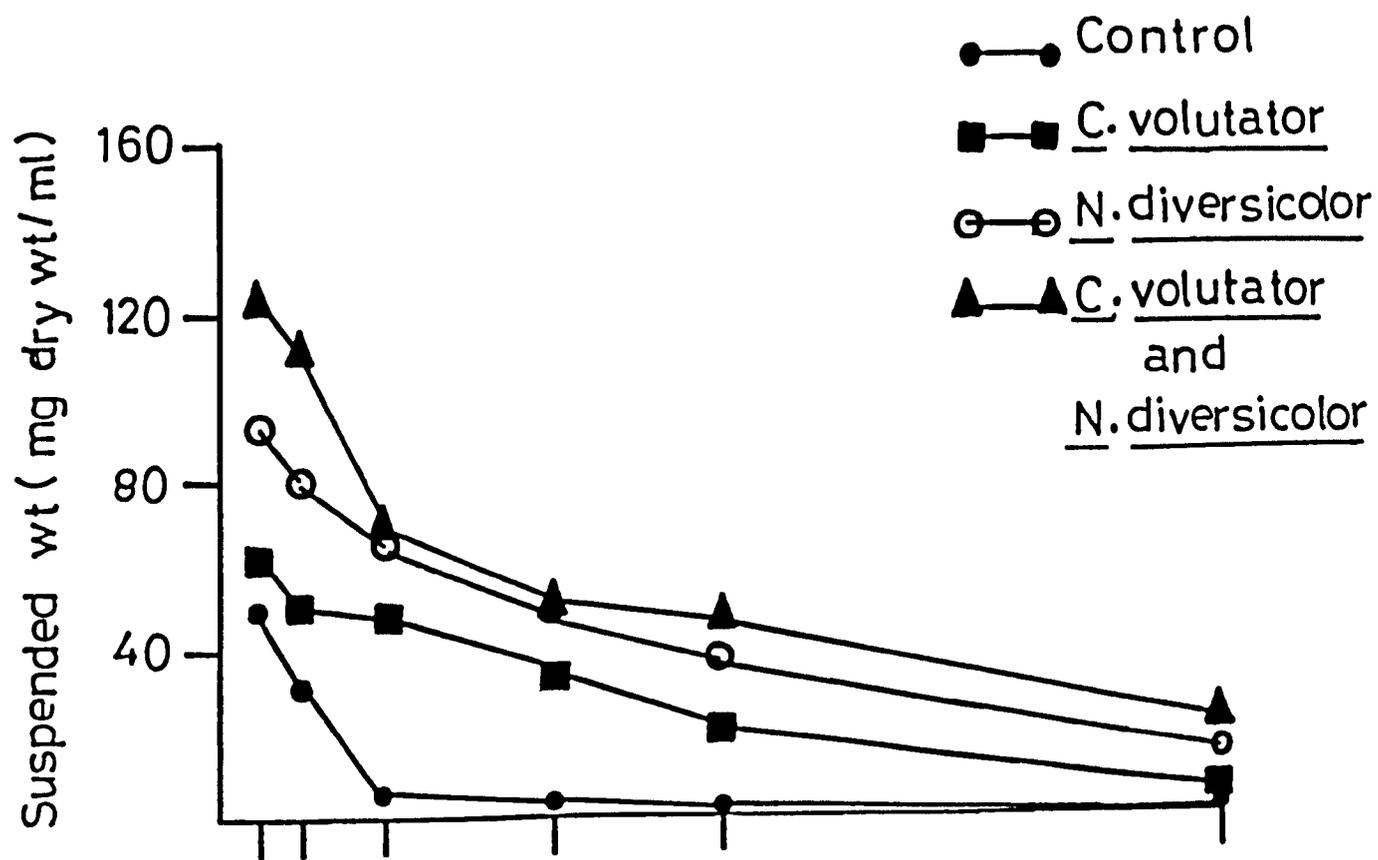


TABLE 51. Statistical analysis of suspended sediment (mg/ml) in experiments testing the effect of animal secretions on sedimentation (Langbank sediment:Langbank species). Six 1 x 4 one-way analyses of variance each comparing control sediment (no animals), sediment containing C. volutator secretions, sediment containing N. diversicolor secretions and sediment containing both species' secretions.

| Time (secs) | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|-------------|---------|----------------|--------------|-----|-----------|-----------------------------|
| 5 | Main | 24782.1 | 8261.0 | 3 | 1978692.1 | P < 0.001 ***** |
| | Error | 0.06680 | 0.004175 | 16 | | |
| | Total | 24783.2 | | 19 | | |
| 10 | Main | 19525.4 | 6508.5 | 3 | 1535017.3 | P < 0.001 ***** & |
| | Error | 0.06784 | 0.004240 | 16 | | |
| | Total | 19525.5 | | 19 | | |
| 20 | Main | 21626.65 | 7208.9 | 3 | 2425180.9 | P < 0.001 ***** |
| | Error | 0.04756 | 0.002973 | 16 | | |
| | Total | 21626.6 | | 19 | | |
| 40 | Main | 27379.1 | 9126.4 | 3 | 1558562.6 | P < 0.001 ***** |
| | Error | 0.09369 | 0.005856 | 16 | | |
| | Total | 27379.2 | | 19 | | |
| 60 | Main | 15399.6 | 5133.2 | 3 | 232923.9 | P < 0.001 ***** |
| | Error | 0.3526 | 0.02204 | 16 | | |
| | Total | 15400.0 | | 19 | | |
| 120 | Main | 7852.7 | 2617.6 | 3 | 591791.7 | P < 0.001 ***** |
| | Error | 0.07077 | 0.004423 | 16 | | |
| | Total | 7852.8 | | 19 | | |

TABLE 52. Statistical analysis of suspended sediment (mg/ml) in experiments testing the effect of animal secretions on sedimentation (Rockware sediment:Langbank species). Six 1 x 4 one-way analyses of variance each comparing control sediment (no animals), sediment containing C. volutator secretions, sediment containing N. diversicolor secretions and sediment containing both species' secretions.

| Time (secs) | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|-------------|---------|----------------|--------------|-----|-----------|------------------------|
| 5 | Main | 16007.2 | 5335.7 | 3 | 1730978.0 | P < 0.001 ***** |
| | Error | 0.04932 | 0.003083 | 16 | | |
| | Total | 16007.3 | | 19 | | |
| 10 | Main | 18646.1 | 6215.4 | 3 | 1494074.8 | P < 0.001 ***** |
| | Error | 0.06656 | 0.004160 | 16 | | |
| | Total | 18646.1 | | 19 | | |
| 20 | Main | 12729.9 | 4243.3 | 3 | 1184243.3 | P < 0.001 ***** |
| | Error | 0.05733 | 0.003583 | 16 | | |
| | Total | 12729.9 | | 19 | | |
| 40 | Main | 6852.8 | 2284.3 | 3 | 288304.7 | P < 0.001 ***** |
| | Error | 0.12677 | 0.007923 | 16 | | |
| | Total | 6852.9 | | 19 | | |
| 60 | Main | 6188.9 | 2063.0 | 3 | 1408.2 | P < 0.001 ***** |
| | Error | 23.44 | 1.465 | 16 | | |
| | Total | 6212.3 | | 19 | | |
| 120 | Main | 1286.8 | 428.9 | 3 | 100467.2 | P < 0.001 ***** |
| | Error | 0.06831 | 0.004269 | 16 | | |
| | Total | 1286.9 | | 19 | | |

TABLE 53. Statistical parameters obtained and calculated from transformed data presented in Figure 42 (lower plot). Initial sediment concentration (mg dry weight/ml), and time for 50% and 95% sedimentation were predicted from the straight line transformation ($\ln y = \ln a + bx$) derived from the curve line ($y = ae^{bx}$). (LBM = Langbank sediment.)

| Experiment | R | T | Probability | Slope (b) | Predicted initial sediment concentration (y intercept = $\ln a$) | | x_{50} (secs) | x_{95} (secs) |
|--|---------|-------|-------------|--------------|---|----------|--------------------|--------------------|
| | | | | | a | $\ln(a)$ | | |
| Control | -0.8904 | 10.35 | $P < 0.001$ | -0.03066 | 37.66 | 3.629 | 22.61 | 97.72 |
| LBM with <u>C. volutator</u> | -0.9743 | 22.89 | $P < 0.001$ | -0.01167 | 87.11 | 4.467 | 59.39 | 256.7 |
| LBM with <u>N. diversicolor</u> | -0.9904 | 37.91 | $P < 0.001$ | -0.008438 | 121.3 | 4.798 | 80.14 | 350.1 |
| LBM with <u>C. volutator</u> and <u>N. diversicolor</u> | -0.9699 | 21.08 | $P < 0.001$ | -0.008547 | 135.1 | 4.906 | 82.09 | 355.5 |

TABLE 54. Statistical parameters obtained and calculated from transformed data presented in Figure 43 (lower plot). Initial sediment concentration (mg dry weight/ml), and time for 50% and 95% sedimentation were predicted from the straight line transformation ($\ln y = \ln a + bx$) derived from the curve line ($y = ae^{bx}$). (RWS = Rockware sediment.)

| Experiment | R | T | Probability | Slope (b) | Predicted initial sediment concentration (y intercept = $\ln a$) | | x_{50} (secs) | x_{95} (secs) |
|--|---------|-------|-------------|--------------|---|----------|--------------------|--------------------|
| | | | | | a | $\ln(a)$ | | |
| Control | -0.8759 | 9.606 | $P < 0.001$ | -0.03074 | 24.73 | 3.208 | 22.55 | 97.46 |
| RWS with <u>C. volutator</u> | -0.9951 | 53.26 | $P < 0.001$ | -0.01786 | 67.10 | 4.206 | 38.81 | 167.7 |
| RWS with <u>N. diversicolor</u> | -0.9977 | 77.88 | $P < 0.001$ | -0.01528 | 94.07 | 4.544 | 45.36 | 196.1 |
| RWS with <u>C. volutator</u> and <u>N. diversicolor</u> | -0.9601 | 18.17 | $P < 0.001$ | -0.01397 | 110.7 | 4.707 | 49.61 | 214.5 |

2. Effect of Ardmore species (*P. elegans*, *S. armiger* and *A. marina*) on sedimentation of Ardmore and Rockware sediment

The results of this part are shown in Figures 44 and 45 and Appendix III, Table 4, p. 520. Control sediment sediments very quickly, followed by sediment treated with *P. elegans* secretions and sediment treated with *A. marina* secretions. However, *S. armiger* has no effect on sedimentation of either Ardmore or Rockware sediments. These conclusions are substantiated by the following statistical analyses.

A series of 1 x 4 one-way analyses of variance were applied to the suspended weights (mg/ml) of the control sediment, the sediment containing *P. elegans* secretions, the sediment containing *S. armiger* secretions and the sediment containing *A. marina* secretions. These anovars were carried out for each of the six time intervals. The first six anovars were conducted on Ardmore sediment containing Ardmore species (Table 55). The second six anovars were conducted on Rockware sediment containing Ardmore species (Table 56). The results from all the twelve one-way anovars were highly significant ($P < 0.001$).

As previously the suspended weights (mg/ml) were transformed to \ln values (Appendix III, Table 5, p. 521) to obtain straight lines of the form $\ln y = \ln a + bx$, where $\ln y = \ln$ (mg dry weight/ml) and x equals time (secs). These lines are shown in the lower half of Figures 44 and 45.

As expected, animal secretions increased the 50% (x_{50}) and the 95% (x_{95}) times of both sediment types (cf. one-way anovars described above). However, in contrast with the Langbank species, the x_{50} and x_{95} times of the Rockware and Ardmore sediments are approximately the same for the control and for the sediments containing secretions (Tables 57 and 58). This similarity probably reflects the similarity of Ardmore and Rockware sediments (see Section I, Discussion, Table 18, p. 144).

Figure 44

Relationships between time intervals (secs) and suspended weights (mg dry weight/ml) of Ardmore sediment. Weights obtained from the untreated (control) sediment and the treated sediment with animal secretions, P. elegans (■—■), S. armiger (O—O), and A. marina (▲—▲). The upper plot shows the original suspended weights, while the lower plot shows the transformed data (ln of suspended weight). Each point is the mean of five replicate samples. Standard deviations are not shown because they are too small to plot. The means and standard deviations of the original data are given in Appendix III, Table 4, p. 520.

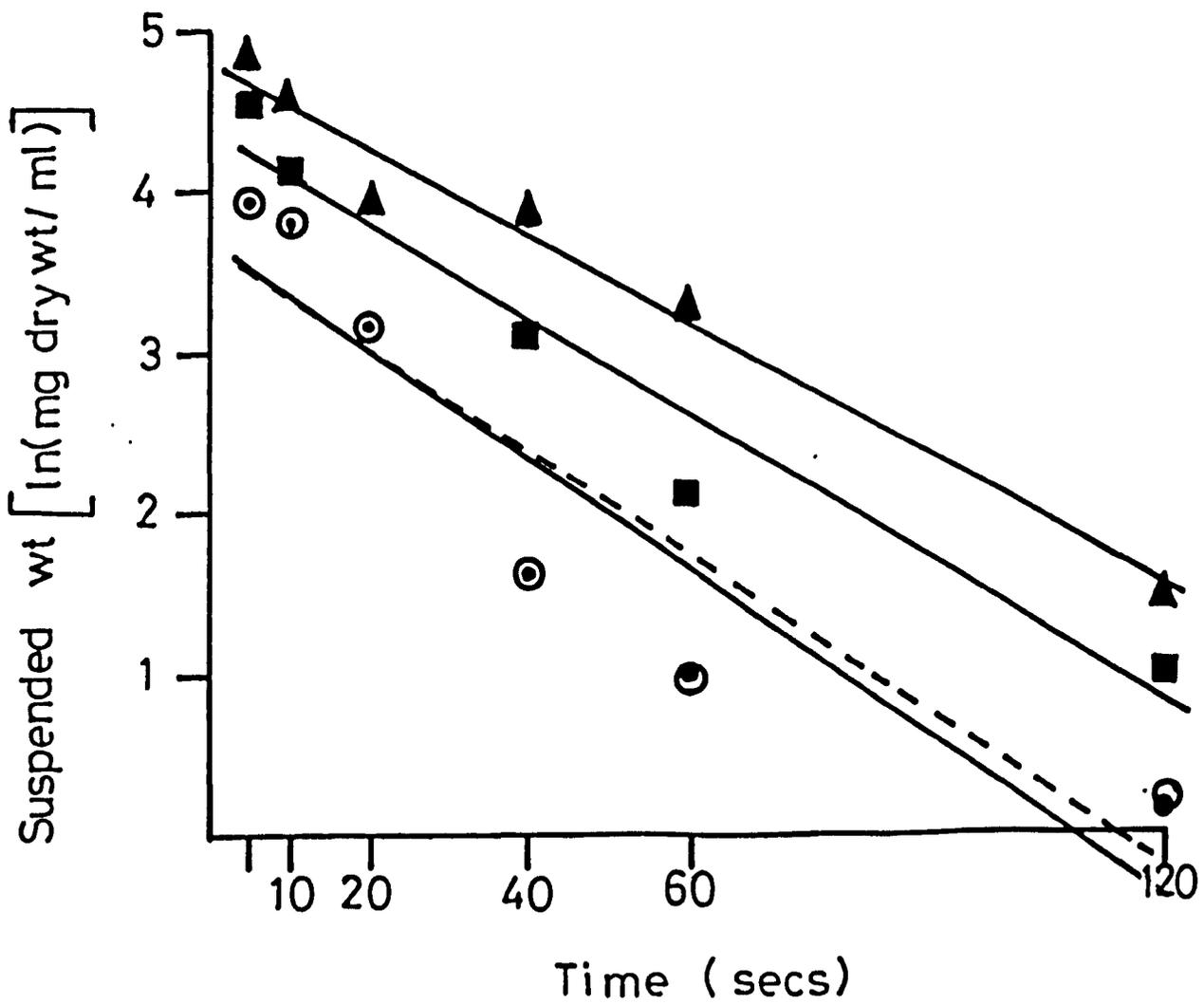
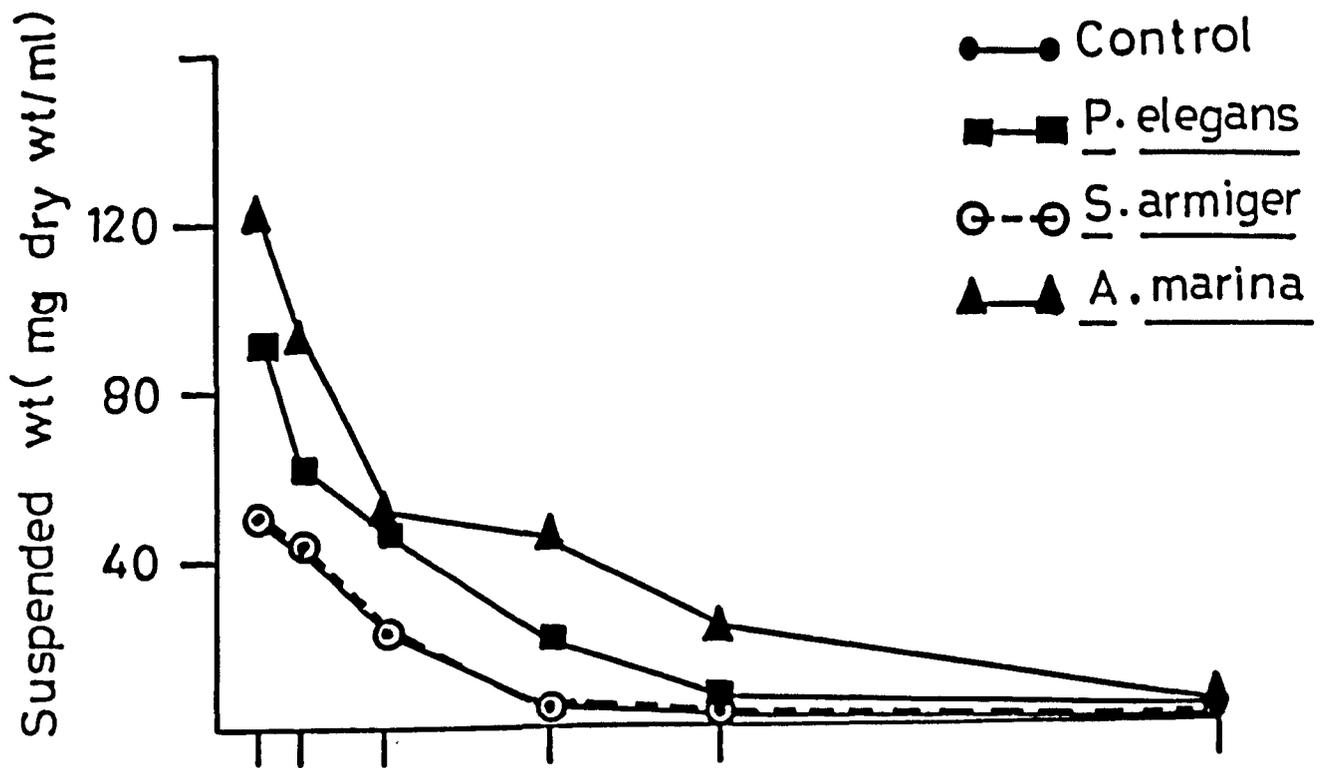


Figure 45

Relationships between time intervals (secs) and suspended weights (mg dry weight/ml) of Rockware sediment. Weights obtained from the untreated (control) sediment and the treated sediment with animal secretions, P. elegans (■—■), S. armiger (○—○), and A. marina (▲—▲). The upper plot shows the original suspended weights, while the lower plot shows the transformed data (ln of suspended weight). Each point is the mean of five replicate samples. Standard deviations are not shown because they are too small to plot. The means and standard deviations of the original data are given in Appendix III, Table 4, p. 520.

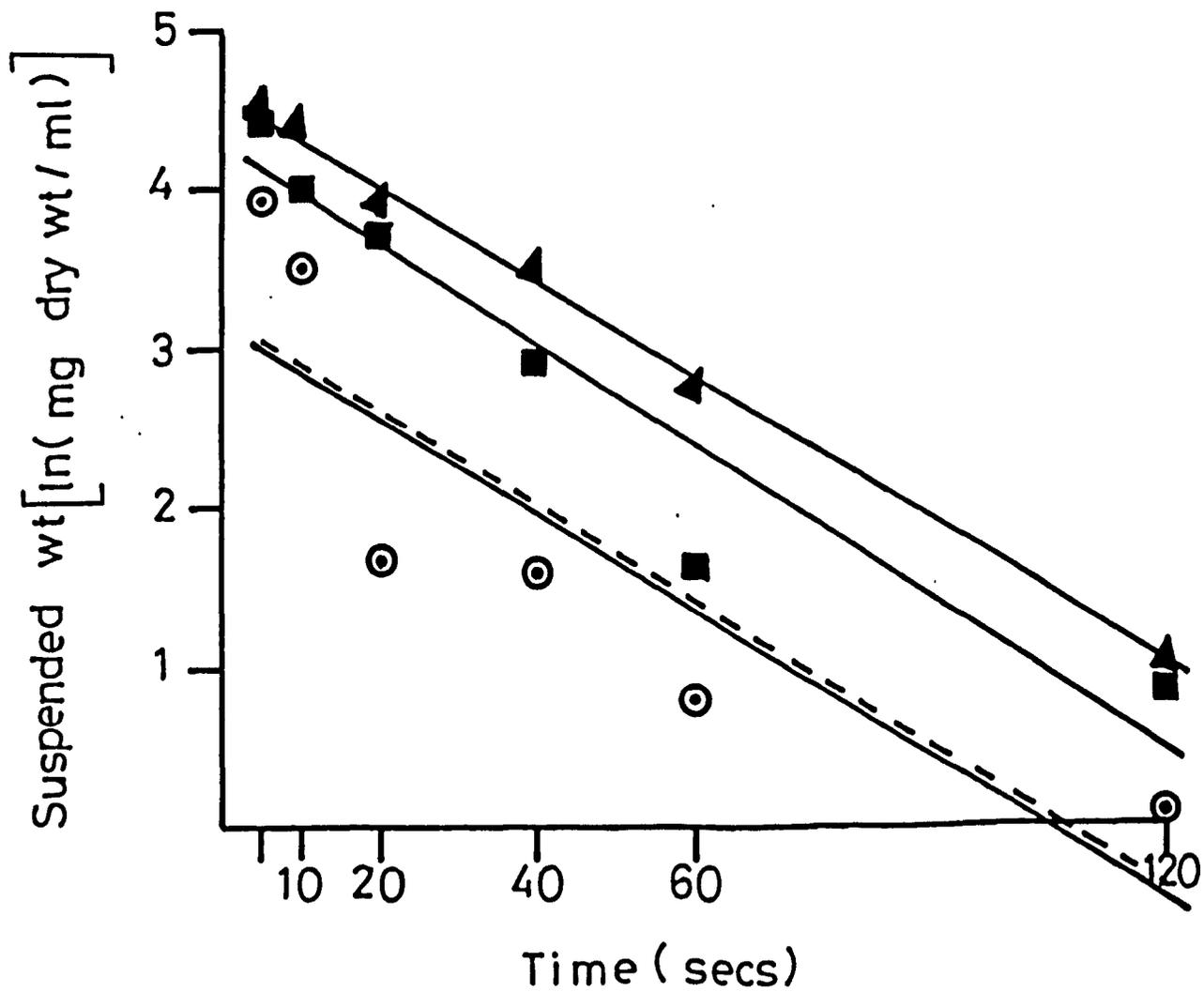
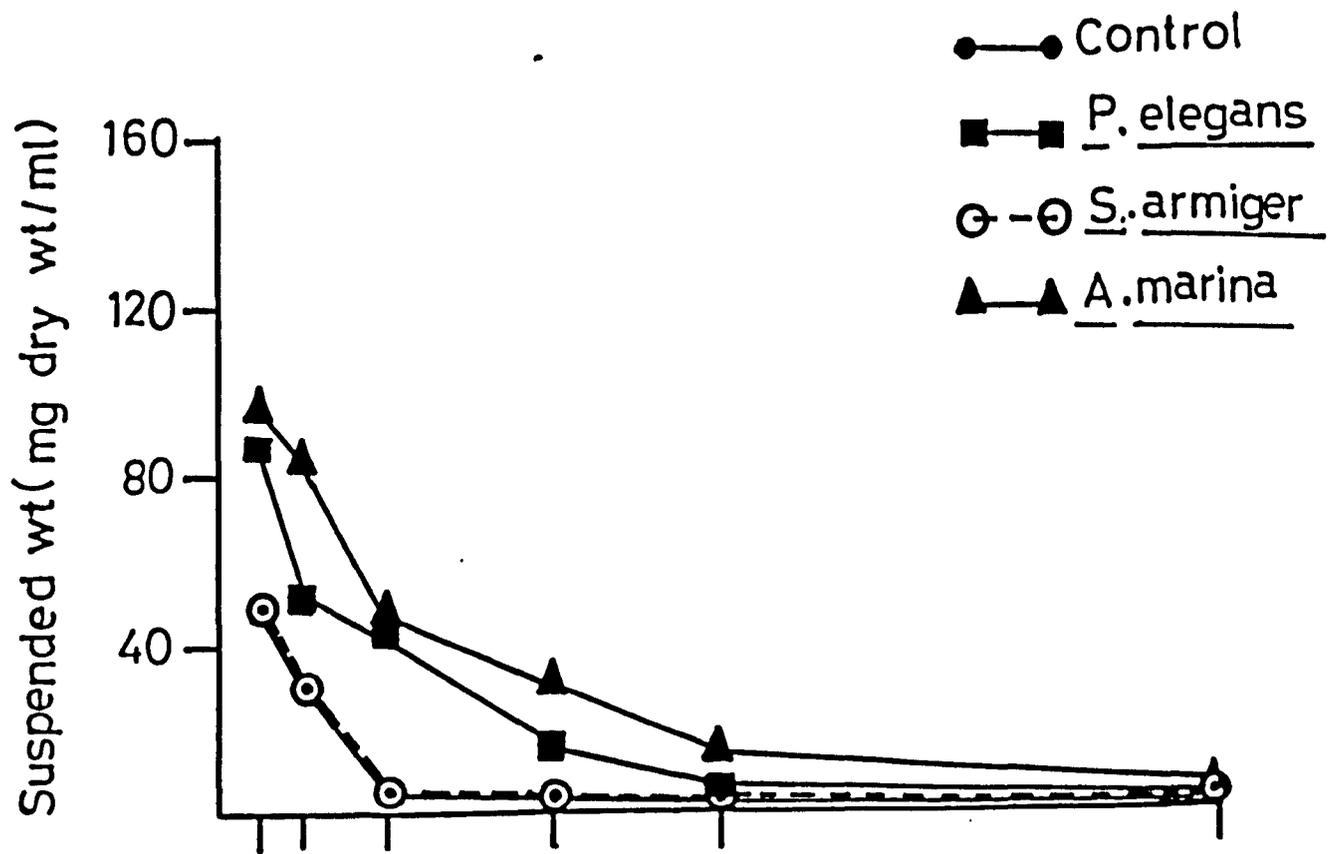


TABLE 55. Statistical analysis of suspended sediment (mg/ml) in experiments testing the effect of animal secretions on sedimentation (Ardmore, sediment:Ardmore species). Six 1 x 4 one-way analyses of variance each comparing control sediment (no animals), sediment containing P. elegans secretions, sediment containing S. armiger secretions and sediment containing A. marina secretions.

| Time (secs) | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|-------------|---------|----------------|--------------|-----|----------|------------------------|
| 5 | Main | 19187.9 | 6398.0 | 3 | 491809.2 | P < 0.001 ***** |
| | Error | 0.2081 | 0.01301 | 16 | | |
| | Total | 19188.1 | | 19 | | |
| 10 | Main | 8129.6 | 1709.9 | 3 | 211.8 | P < 0.001 ***** |
| | Error | 204.7 | 12.79 | 16 | | |
| | Total | 8334.3 | | 19 | | |
| 20 | Main | 3638.1 | 1212.7 | 3 | 244.4 | P < 0.001 ***** |
| | Error | 79.39 | 4.962 | 16 | | |
| | Total | 3717.5 | | 19 | | |
| 40 | Main | 6272.2 | 2090.7 | 3 | 152488.9 | P < 0.001 ***** |
| | Error | 0.2194 | 0.01371 | 16 | | |
| | Total | 6272.4 | | 19 | | |
| 60 | Main | 1902.8 | 634.3 | 3 | 43224.8 | P < 0.001 ***** |
| | Error | 0.2348 | 0.01467 | 16 | | |
| | Total | 1903.04 | | 19 | | |
| 120 | Main | 34.27 | 11.42 | 3 | 888.3 | P < 0.001 ***** |
| | Error | 0.2058 | 0.01286 | 16 | | |
| | Total | 34.48 | | 19 | | |

TABLE 56. Statistical analysis of suspended sediment (mg/ml) in experiments testing the effect of animal secretions on sedimentation (Rockware sediment:Ardmore species). Six 1 x 4 one-way analyses of variance each comparing control sediment (no animals), sediment containing P. elegans secretions, sediment containing S. armiger secretions and sediment containing A. marina secretions.

| Time (secs) | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|-------------|---------|----------------|--------------|-----|----------|------------------------|
| 5 | Main | 9722.7 | 3240.9 | 3 | 37673.8 | P < 0.001 ***** |
| | Error | 1.3764 | 0.08603 | 16 | | |
| | Total | 9724.04 | | 19 | | |
| 10 | Main | 8955.7 | 2985.2 | 3 | 696265.2 | P < 0.001 ***** |
| | Error | 0.0686 | 0.004288 | 16 | | |
| | Total | 8955.8 | | 19 | | |
| 20 | Main | 8819.7 | 2939.9 | 3 | 602592.1 | P < 0.001 ***** |
| | Error | 0.07806 | 0.004879 | 16 | | |
| | Total | 8819.8 | | 19 | | |
| 40 | Main | 2707.0 | 902.3 | 3 | 240420.2 | P < 0.001 ***** |
| | Error | 0.06005 | 0.003753 | 16 | | |
| | Total | 2707.04 | | 19 | | |
| 60 | Main | 637.4 | 212.5 | 3 | 87688.4 | P < 0.001 ***** |
| | Error | 0.03877 | 0.002423 | 16 | | |
| | Total | 637.5 | | 19 | | |
| 120 | Main | 12.60 | 4.2009 | 3 | 1867.1 | P < 0.001 ***** |
| | Error | 0.03600 | 0.00225 | 16 | | |
| | Total | 12.64 | | 19 | | |

TABLE 57. Statistical parameters obtained and calculated from transformed data presented in Figure 44 (lower plot). Initial sediment concentration (mg dry weight/ml), and time for 50% and 95% sedimentation were predicted from the straight line transformation ($\ln y = \ln a + bx$) derived from the curve line ($y = ae^{bx}$). (ARD = Ardmore sediment.)

| Experiment | R | T | Probability | Slope (b) | Predicted initial sediment concentration (y intercept = $\ln a$) | | x_{50} (secs) | x_{95} (secs) |
|-------------------------------|---------|-------|-------------|--------------|---|----------|--------------------|--------------------|
| | | | | | a | $\ln(a)$ | | |
| Control | -0.9221 | 12.61 | $P < 0.001$ | -0.03411 | 39.91 | 3.687 | 20.82 | 87.83 |
| ARD with <u>P. elegans</u> | -0.9763 | 23.87 | $P < 0.001$ | -0.03028 | 84.66 | 4.439 | 22.89 | 98.94 |
| ARD with <u>S. armiger</u> | -0.9227 | 12.66 | $P < 0.001$ | -0.03321 | 40.29 | 3.696 | 20.89 | 90.21 |
| ARD with <u>A. marina</u> | -0.9888 | 35.06 | $P < 0.001$ | -0.02744 | 123.8 | 4.819 | 25.26 | 109.2 |

TABLE 58. Statistical parameters obtained and calculated from transformed data presented in Figure 45 (lower plot). Initial sediment concentration (mg dry weight/ml), and time for 50% and 95% sedimentation were predicted from the straight line transformation ($\ln y = \ln a + bx$) derived from the curve line ($y = ae^{bx}$). (RWS = Rockware sediment.)

| Experiment | R | T | Probability | Slope (b) | Predicted initial sediment concentration (y intercept = $\ln a$) | | x_{50} (secs) | x_{95} (secs) |
|-------------------------------|---------|-------|-------------|--------------|---|----------|--------------------|--------------------|
| | | | | | a | $\ln(a)$ | | |
| Control | -0.8759 | 9.606 | $P < 0.001$ | -0.03074 | 24.73 | 3.208 | 20.55 | 87.53 |
| RWS with <u>P. elegans</u> | -0.9546 | 16.96 | $P < 0.001$ | -0.03189 | 73.65 | 4.299 | 22.73 | 93.95 |
| RWS with <u>S. armiger</u> | -0.8726 | 9.453 | $P < 0.001$ | -0.03031 | 24.61 | 3.203 | 20.87 | 90.15 |
| RWS with <u>A. marina</u> | -0.9977 | 77.88 | $P < 0.001$ | -0.03052 | 106.3 | 4.666 | 24.71 | 98.17 |

3. Statistical comparisons between the effect of Langbank species and Ardmore species on sedimentation of Rockware sediment

The effects of C. volutator and N. diversicolor (Langbank species) and P. elegans and A. marina (Ardmore species) were compared by a series of 1 x 2 one-way analyses of variance, in which pairs of species were compared in turn. This resulted in thirty-six one-way anovars, six at each time interval (Appendix III, Tables 6-11, pp. 522-527).

Highly significant differences ($P < 0.001$) were found in all the comparisons. This means that each species had significantly different effects on sedimentation of Rockware sediment.

The effects of the species were ranked (Table 59) and the ranks show that in general (sum of ranks) the secretions of N. diversicolor have the greatest effect on decreasing sedimentation, followed by those of A. marina, C. volutator and P. elegans. However, some interesting changes in ranking occurred with time.

At 5 and 10 seconds, A. marina was ranked (1) followed by N. diversicolor, P. elegans and then C. volutator. This means that secretions of A. marina and N. diversicolor have more effect on sedimentation than the other two species at the beginning of the experiment. However, at 40, 60 and 120 seconds, N. diversicolor was ranked (1) followed by C. volutator, A. marina and P. elegans. Hence, N. diversicolor and C. volutator have greatest effects on decreasing sedimentation towards the end of the experiment.

These observations mean that A. marina followed by N. diversicolor have the greatest effect in decreasing sedimentation of large particles (5, 10 seconds) while N. diversicolor followed by C. volutator have the greatest effect on decreasing sedimentation of small particles (40, 60, 120 seconds) (see Discussion, p. 420).

TABLE 59. Comparison of the effect of secretions of C. volutator, N. diversicolor, P. elegans and A. marina on sedimentation of Rockware sediment. The ranks were obtained by comparing the four mean suspended weights (Appendix III, Table 2, rows 2 and 3, and Appendix III, Table 4, rows 2 and 4) for each time interval. The species with the highest mean value was ranked (1) and the species with the lowest value (4). Ranking in columns. *Particle sizes measured in a subsequent experiment (Appendix III, Table 30, p. 546).

| Species | Ranks at successive times | | | | | | Sum of ranks |
|------------------------------|---------------------------|---------|---------|---------|---------|----------|--------------|
| | 5 secs | 10 secs | 20 secs | 40 secs | 60 secs | 120 secs | |
| <u>Arenicola marina</u> | 1 | 1 | 2 | 3 | 3 | 3 | 13 |
| <u>Nereis diversicolor</u> * | 2 | 2 | 1 | 1 | 1 | 1 | 8 |
| <u>Pygospio elegans</u> | 3 | 3 | 4 | 4 | 4 | 4 | 22 |
| <u>Corophium volutator</u> * | 4 | 4 | 3 | 2 | 2 | 2 | 17 |



larger particles*

Intermediate



smaller particles*

4. Scanning electron microscopy of the secretions

Scanning electron microscope photomicrographs of the secretions produced by the different animal species from Langbank and Ardmore are shown in Plates 24-35 and in Plates 36-44.

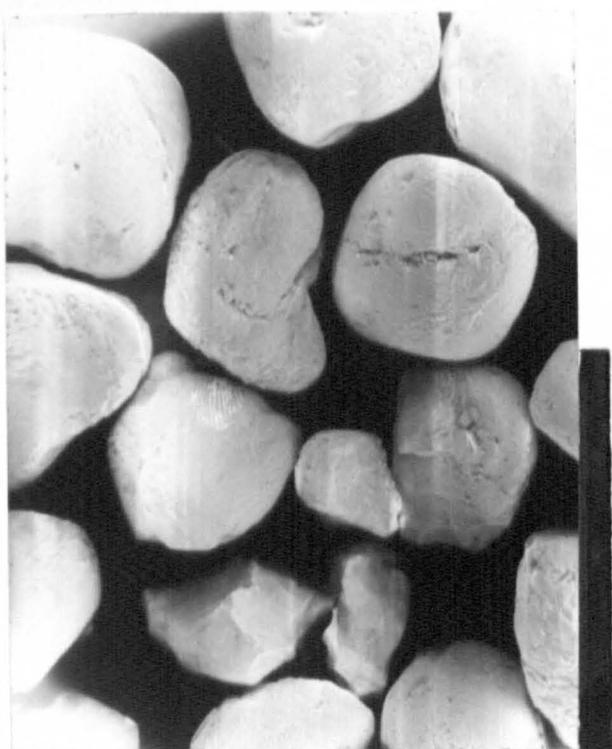
(a) Secretions of Langbank species (C. volutator and N. diversicolor)

At X80 magnification (Plates 24-27), secretions produced by the different species either cover or join the particles, but their structure is not clear. At X320 magnification (Plates 28-31), secretions from C. volutator (Plate 29) and N. diversicolor (Plate 30) are made up of thread-like structures. In addition, leaf-like structures can be seen attached to the secretions of N. diversicolor. In the mixed photographs, secretions from both species are mixed and their structures are not easily distinguishable (Plate 31). At X10,000 magnification (Plates 32-35), the secretions of the two species can be clearly distinguished. Secretions of N. diversicolor (Plate 34) are thinner than those of C. volutator (Plate 33) and the leaf-like structures can be clearly seen. Small beads are obvious along the length of the threads of both species (Plates 33, 34). In the mixed secretions (Plate 35), the leaf-like structures of N. diversicolor secretions are also obvious.

Plates 24-27

Scanning electron microscope photomicrographs of Rockware sediment containing secretions of Langbank species.

Control sediment (Plate 24, X122); C. volutator secretions (Plate 25, X134); N. diversicolor secretions (Plate 26, X106); secretions of both species (Plate 27, X106). Bar lines = 500 μ .



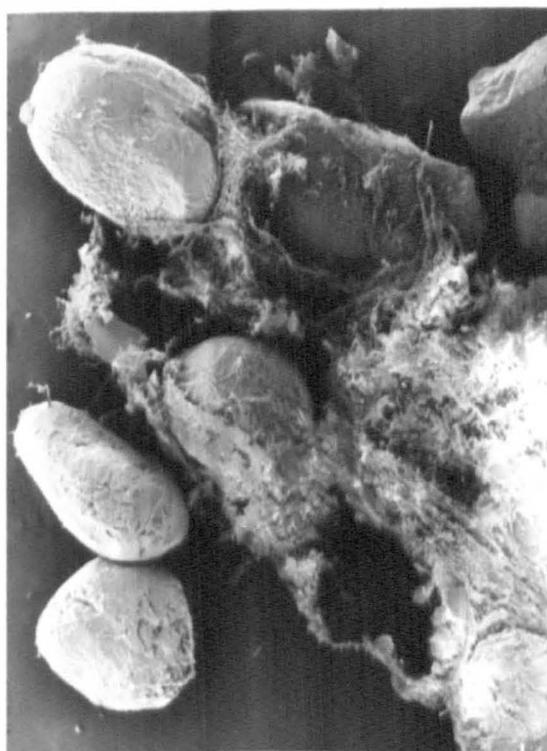
24



25



26



27

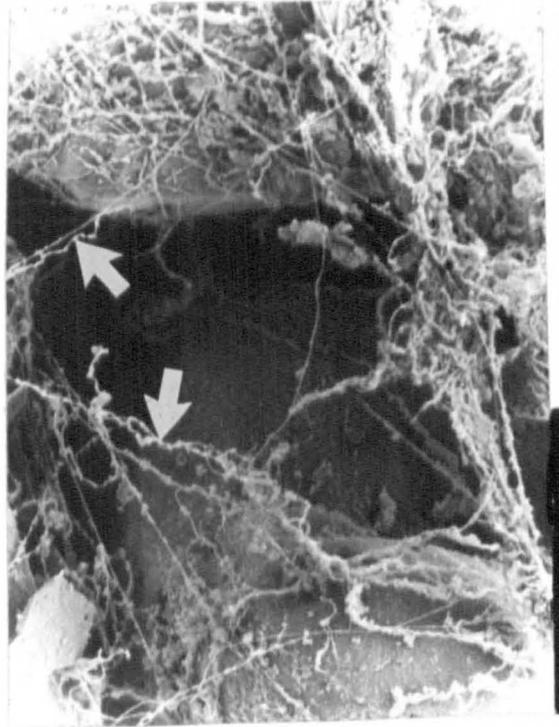
Plates 28-31

Scanning electron microscope photomicrographs of Rockware sediment containing secretions of Langbank species.

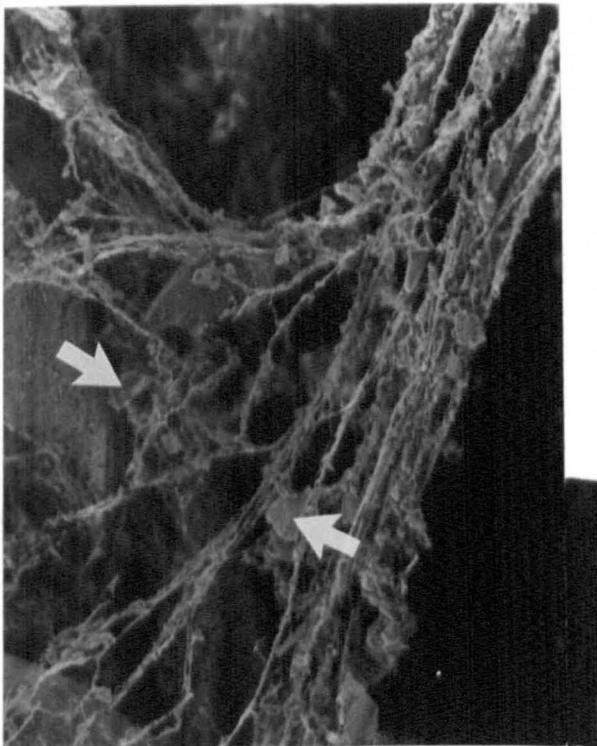
Control sediment (Plate 28, X483); C. volutator secretions (Plate 29, X496); N. diversicolor secretions (Plate 30, X419); secretions of both species (Plate 31, X419). Bar lines = 100 μ .



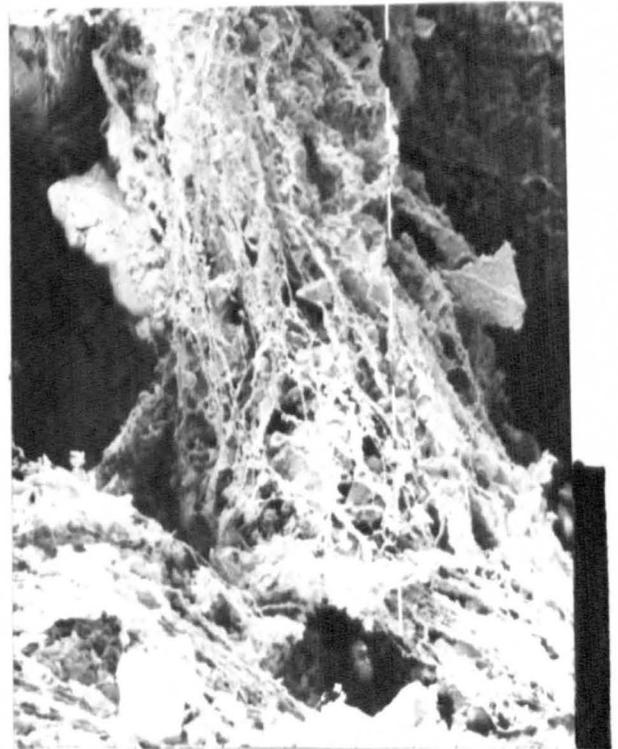
28



29



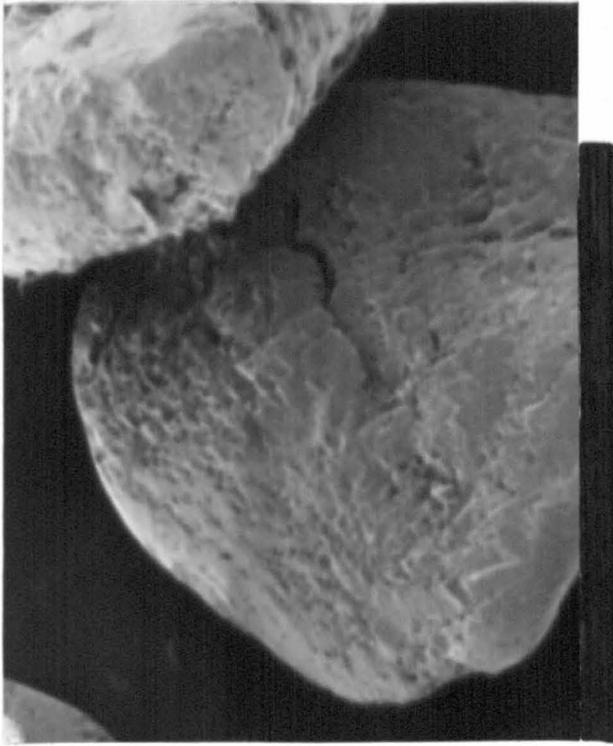
30



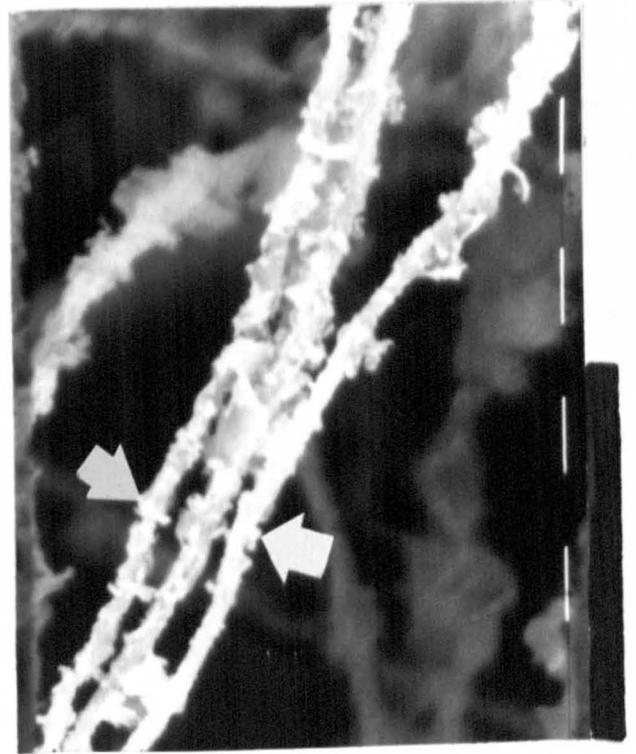
31

Plates 32-35

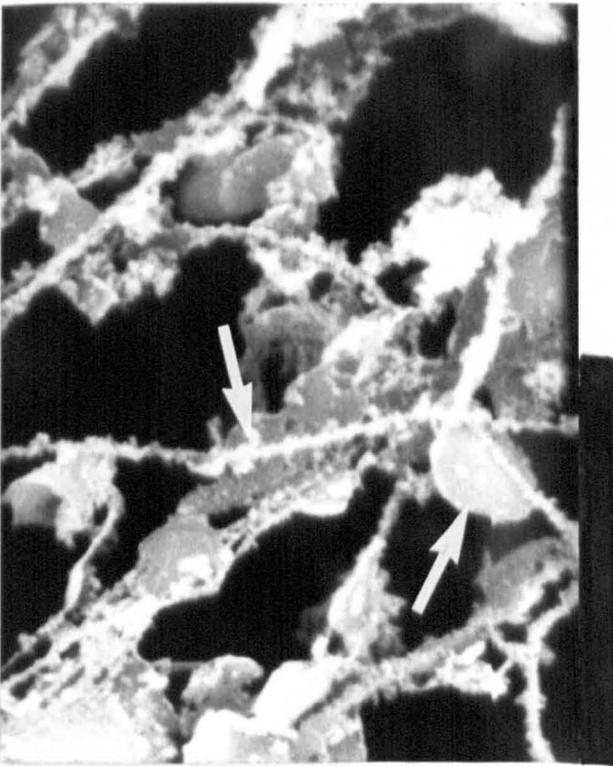
Scanning electron microscope photomicrographs of Rockware sediment containing secretions of Langbank species. Control sediment (Plate 32, X20,000); C. volutator secretions (Plate 33, X12,500); N. diversicolor secretions (Plate 34, X13,200); secretions of both species (Plate 35, X13,000). Bar lines = 5 μ .



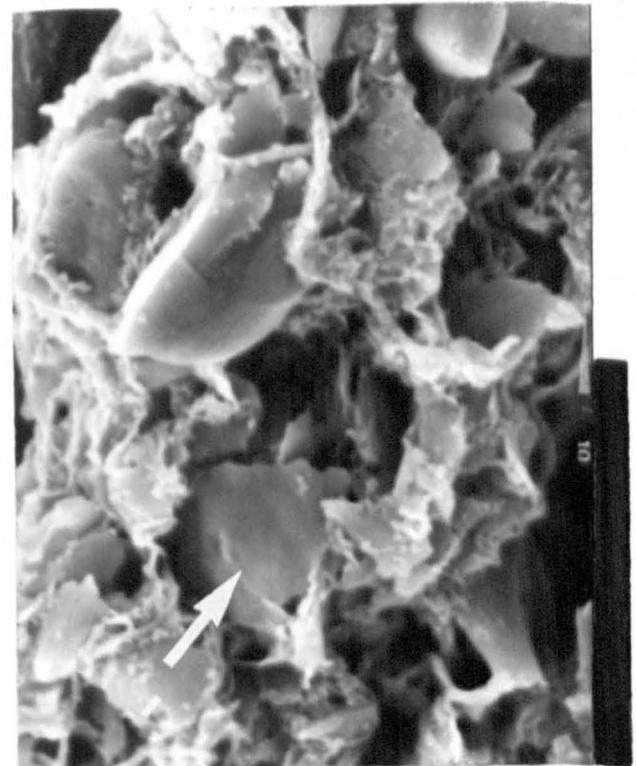
32



33



34



35

(b) Secretions of Ardmere species (P. elegans and A. marina)

At X80 magnification (Plates 36-38), secretions produced by both species look like sheets covering the particles. Banding is obvious in the A. marina secretions (Plate 38). At X320 magnification (Plates 39-41), secretions of P. elegans are sometimes net-like (Plate 40), and those of A. marina are made up of bundles or cords (Plate 41). At X5000 magnification (Plates 42-44), secretions from the two species are clearly distinguishable. The tight net-like structures of P. elegans secretions are very obvious (Plate 43), compared with the more dense sheets of bands or cords in the secretions of A. marina (Plate 44).

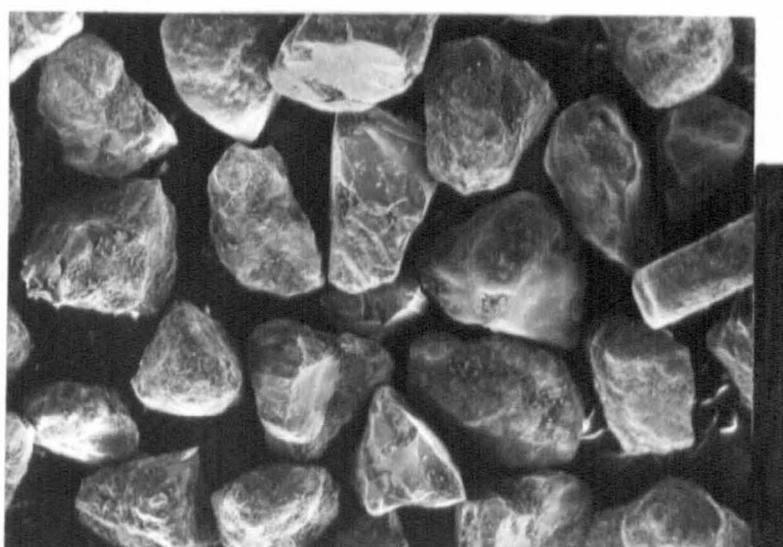
From my observations during sampling and sieving P. elegans, it was obvious that its tubes had a very strong structure as it was difficult to break them apart. At that time, I thought that their resistance to breaking was probably due to the type of secretions involved in the tube construction. These observations are now supported by my scanning electron photomicrographs showing a tight net-like structure holding particles together.

Plates 36-38

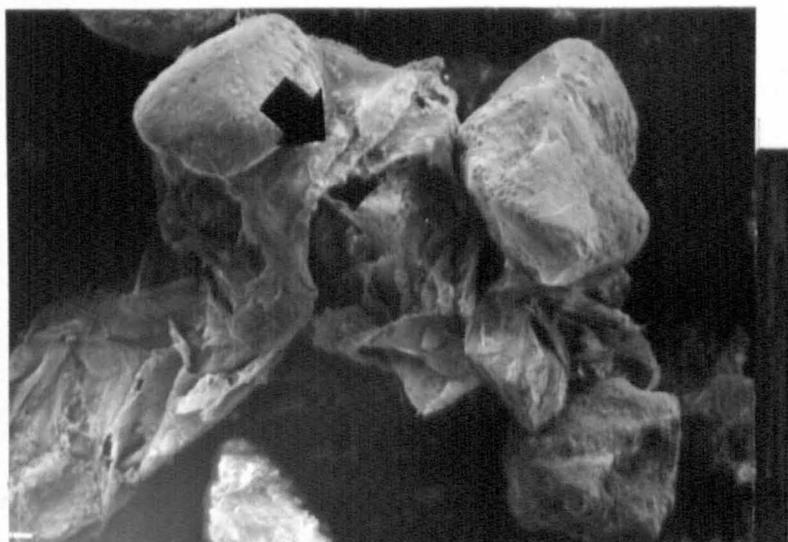
Scanning electron microscope photomicrographs of Rockware sediment containing secretions of Ardmore species.

Control sediment (Plate 36, X115); P. elegans secretions (Plate 37, X118); A. marina secretions (Plate 38, X116).

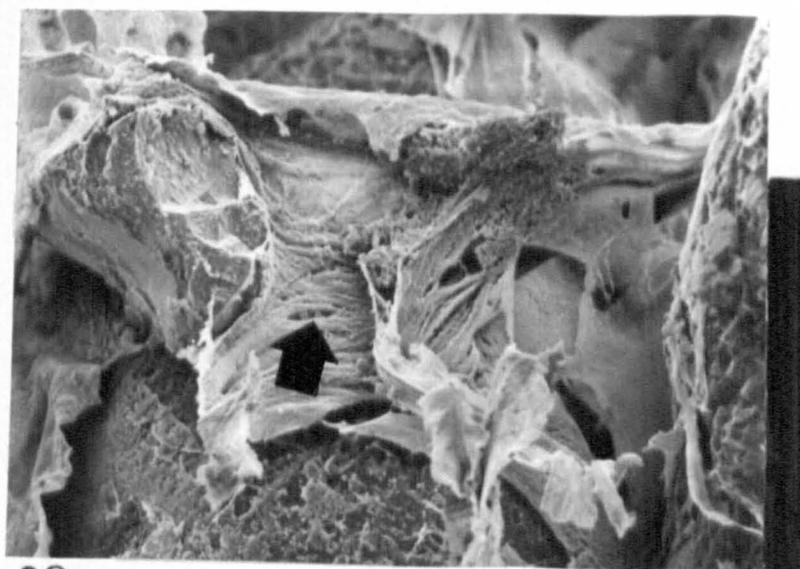
Bar lines = 500 μ .



36



37



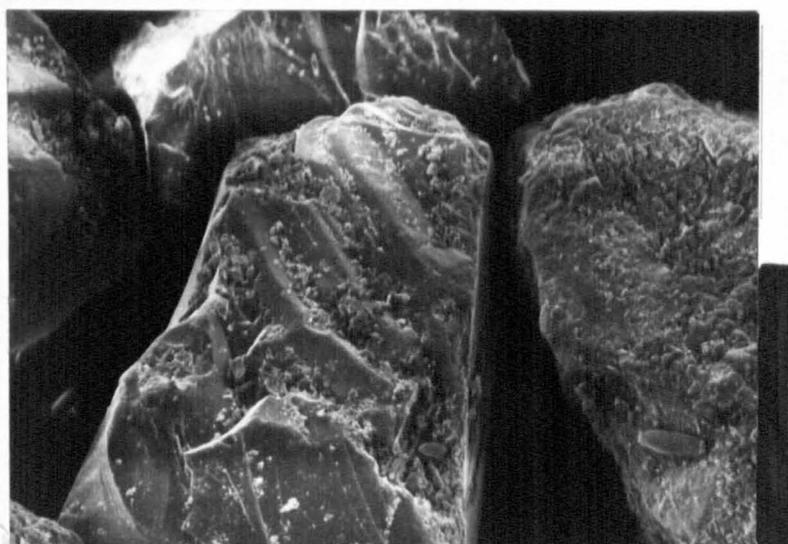
38

Plates 39-41

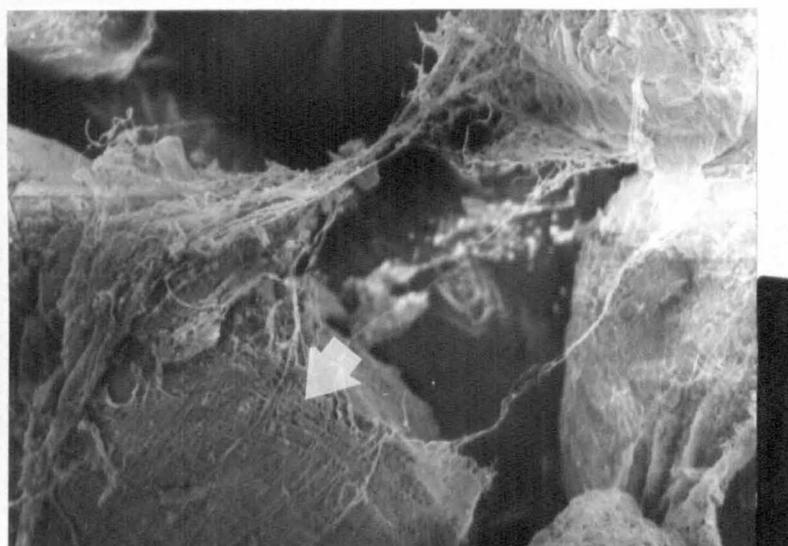
Scanning electron microscope photomicrographs of Rockware sediment containing secretions of Ardmore species.

Control sediment (Plate 39, X451); P. elegans secretions (Plate 40, X426); A. marina secretions (Plate 41, X448).

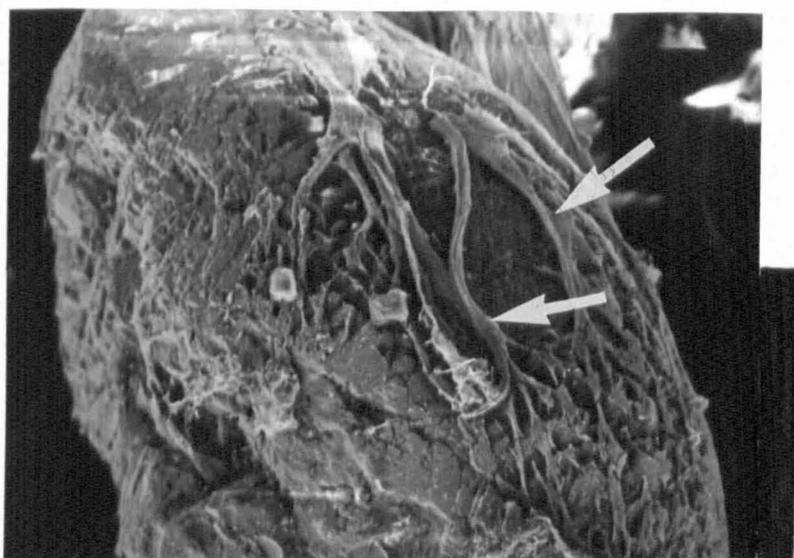
Bar lines = 100 μ .



39



40

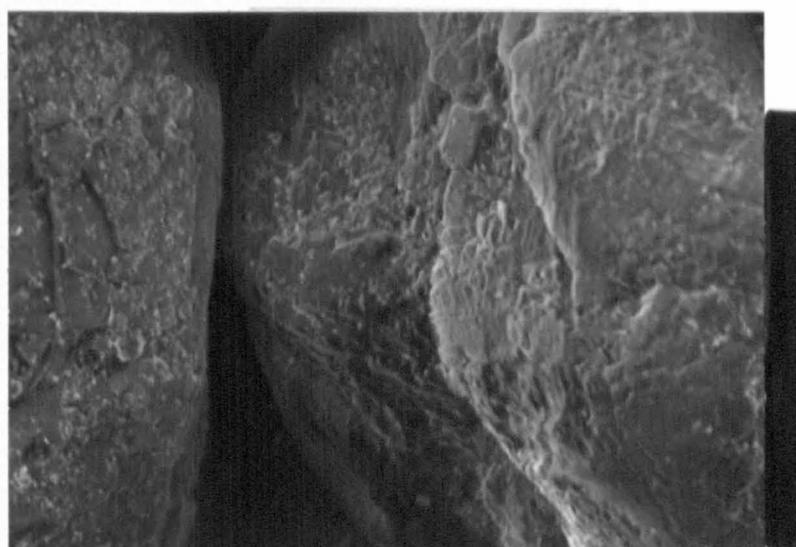


41

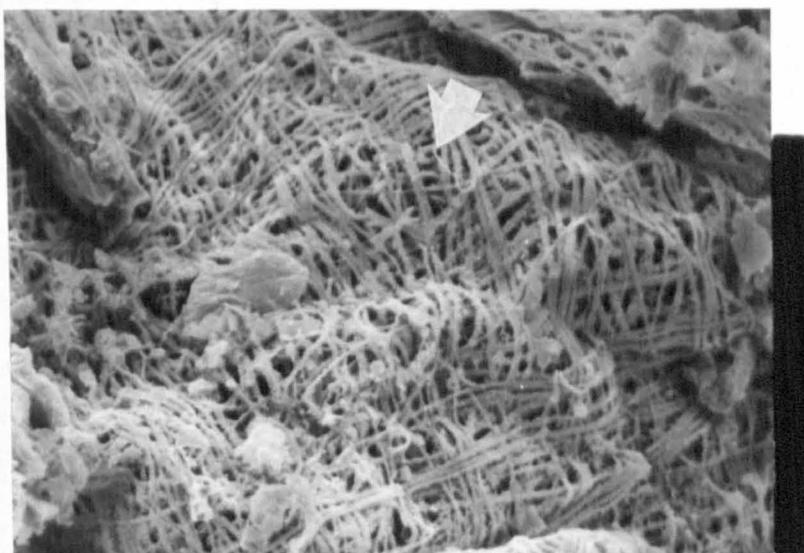
Plates 42-44

Scanning electron microscope photomicrographs of Rockware sediment containing secretions of Ardmore species.

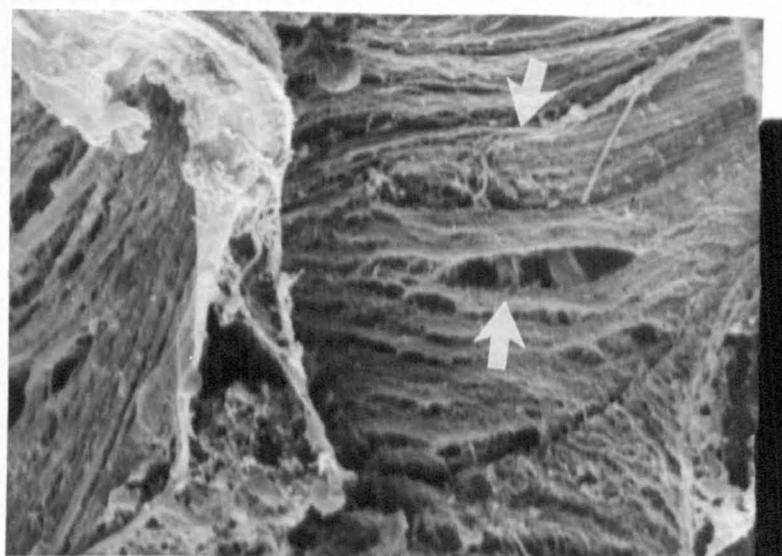
Control sediment (Plate 42, X7250); P. elegans secretions (Plate 43, X6900); A. marina secretions (Plate 44, X7100). Bar lines = 5 μ .



42



43



44

(II) Effect of different enzymes on animal secretions

The results in this section are divided into three parts. The first (1) gives the effect of different enzymes on secretions from C. volutator and N. diversicolor. The second (2) gives the effect of different enzymes on secretions from P. elegans and A. marina. The third (3) gives ranking on enzymes and species in Parts 1 and 2.

1. Effect of different enzymes on C. volutator and N. diversicolor secretions

The results of this part are shown in Figure 46 and Appendix III, Table 13, p. 529. Figure 46 shows the weights of secretions remaining after enzymic digestion as a percentage of the mean of the controls, for C. volutator and N. diversicolor.

The most effective enzymes on C. volutator secretions were α -amylase and trypsin since no secretions remained after treatment with them. They were followed by hyaluronidase, pepsin and lysozyme. The least effective enzyme was lipase.

The most effective enzyme on N. diversicolor secretions was also α -amylase, followed by pepsin, lysozyme, lipase and trypsin. The least effective enzyme was hyaluronidase.

Two one-way analyses of variance were used to analyse the data. These anovars tested differences between the effects of the six different enzymes, each with six levels. The first one-way anovar tested the effect of the different enzymes on C. volutator secretions and the second on N. diversicolor secretions. Both analyses were significant (Table 60). This means that the different enzymes produced

significantly different digestive effects on both species' secretions.

A two-way analysis of variance was then conducted to determine whether the pattern of enzymic digestion was different between the two species. The result (Table 61) showed a significant interaction, thus proving the point.

Figure 46

Effect of enzymes on secretions of Corophium volutator and Nereis diversicolor. The numbers on the x-axis refer to the different enzymes used; (1) α -amylase, (2) hyaluronidase, (3) lipase, (4) lysozyme, (5) pepsin, and (6) trypsin. On the y-axis, the weights of secretions remaining after enzymic digestion are expressed as a per cent weight of the mean of the controls. The different columns (□ and ■) represent two different experiments which were treated as replicates in the statistical analyses.

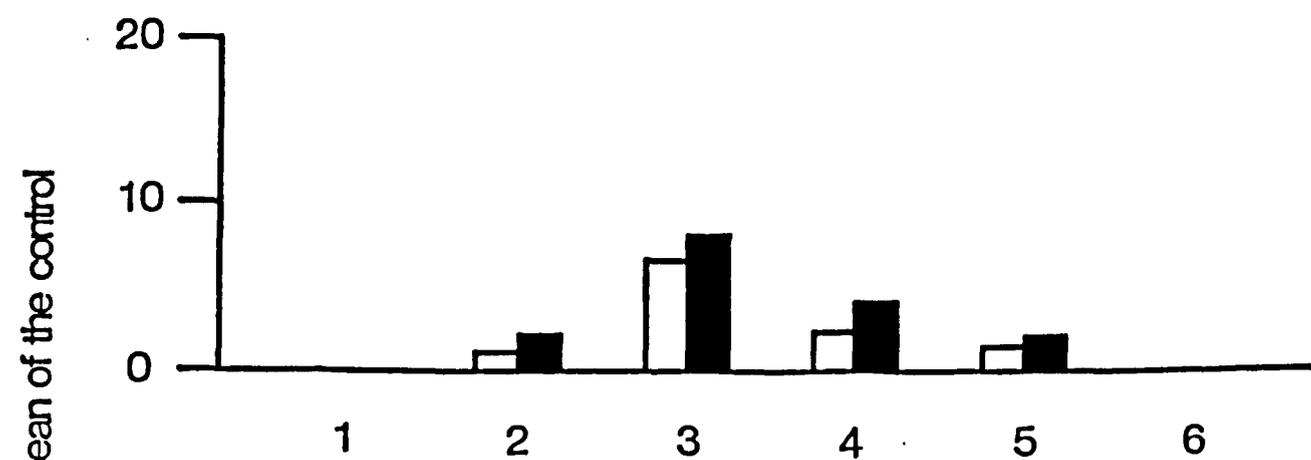
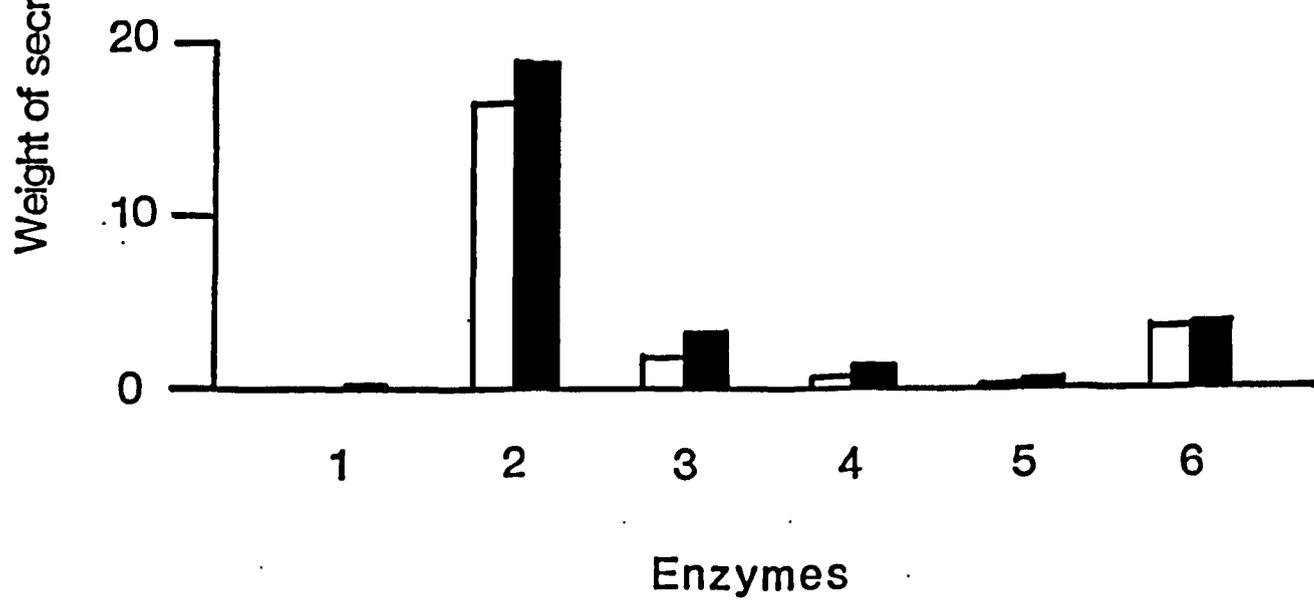
C. volutatorN. diversicolor

TABLE 60. Statistical analysis of the effect of enzymes on C. volutator and N. diversicolor secretions. One-way analyses of variance comparing the effect of the six enzymes on secretions of C. volutator and N. diversicolor. Each anovar has six levels - the six enzymes, and each level contains two readings (i.e. data from the two experiments). The analyses were conducted on the arcsine values in Appendix III, Table 12, p. 528.

| Species | Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|--------------------------------------|---------|----------------|--------------|-----|---------|------------------------|
| <u>C. volutator</u> secretions | Main | 385.8 | 77.17 | 5 | 222.5 | P < 0.001 ***** |
| | Error | 2.080 | 0.3500 | 6 | | |
| | Total | 387.9 | | 11 | | |
| <u>N. diversicolor</u> secretions | Main | 710.5 | 142.1 | 5 | 55.57 | P < 0.001 ***** |
| | Error | 15.34 | 2.560 | 6 | | |
| | Total | 725.8 | | 11 | | |

TABLE 61. Two-way analysis of variance on the effects of enzymic digestion on secretions of C. volutator and N. diversicolor.

| Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|-------------|----------------|--------------|-----|---------|--------------------|
| A | 1759.8 | 1759.8 | 1 | 73.90 | |
| B | 3336.4 | 667.3 | 5 | 28.02 | |
| Interaction | 727.1 | 145.4 | 5 | 6.107 | P < 0.001 ***** |
| Error | 285.7 | 23.81 | 12 | | |
| Total | 6109.1 | | 23 | | |

2. Effect of different enzymes on *P. elegans* and *A. marina* secretions

The results of this part are shown in Figures 47 and 48 and Appendix III, Table 14, p. 530. These figures show the weights of secretions remaining after enzymic digestion as a percentage of the mean of the control for *P. elegans* (Figure 47) and *A. marina* (Figure 48).

The most effective enzyme on *P. elegans* secretions was lipase followed by α -amylase, lysozyme, pepsin and trypsin. The least effective enzyme was hyaluronidase.

The most effective enzyme on *A. marina* secretions was α -amylase followed by pepsin, lipase, lysozyme and hyaluronidase. The least effective enzyme was trypsin.

Two one-way analysis of variance were used to analyse the data. These anovars tested differences between the effects of the six different enzymes, each with six levels. The first one-way anovar tested the effect of the different enzymes on *P. elegans* secretions and the second on *A. marina* secretions. Both were significant (Table 62). This means that the different enzymes produced significantly different effects on both species secretions.

A two-way analysis of variance was then conducted to determine whether the pattern of enzymic digestion was different between the two species. The result (Table 63) showed a significant interaction, thus proving that the pattern was different.

Figure 47

Effect of enzymes on Pygospio elegans secretions. The numbers on the x-axis refer to the different enzymes used; (1) α -amylase, (2) hyaluronidase, (3) lipase, (4) lysozyme, (5) pepsin, and (6) trypsin. On the y-axis, the weights of secretions remaining after enzymic digestion are expressed as a per cent weight of the mean of the controls. The different columns (□ and ■) represent two different experiments which were treated as replicates in the statistical analyses.

Weight of secretion as a % of the mean of the control

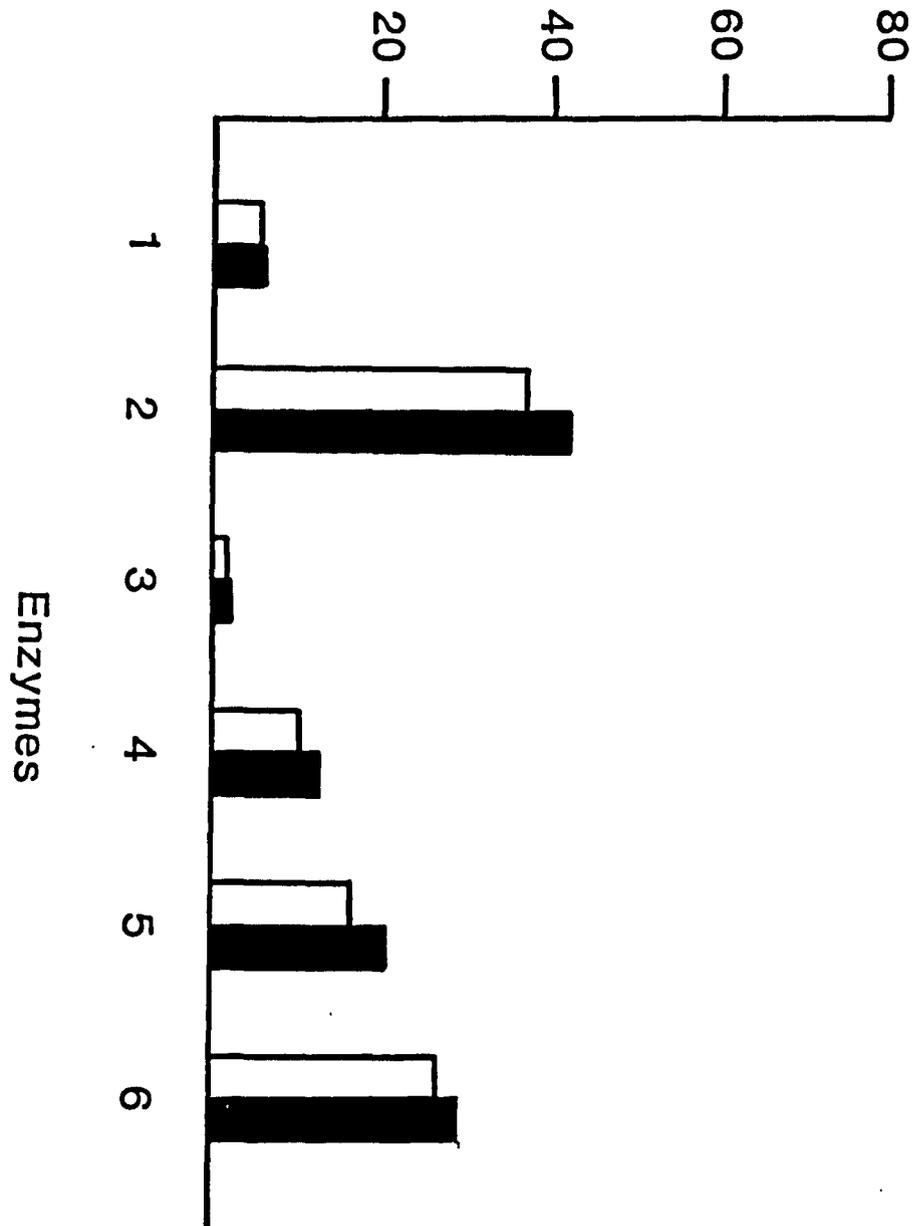


Figure 48

Effect of enzymes on Arenicola marina secretions. The numbers on the x-axis refer to the different enzymes used; (1) α -amylase, (2) hyaluronidase, (3) lipase, (4) lysozyme, (5) pepsin, and (6) trypsin. On the y-axis, the weights of secretions remaining after enzymic digestion are expressed as a per cent weight of the mean of the controls. The different columns (□ and ■) represent two different experiments which were treated as replicates in the statistical analyses.

Weight of secretion as a % of the mean of control

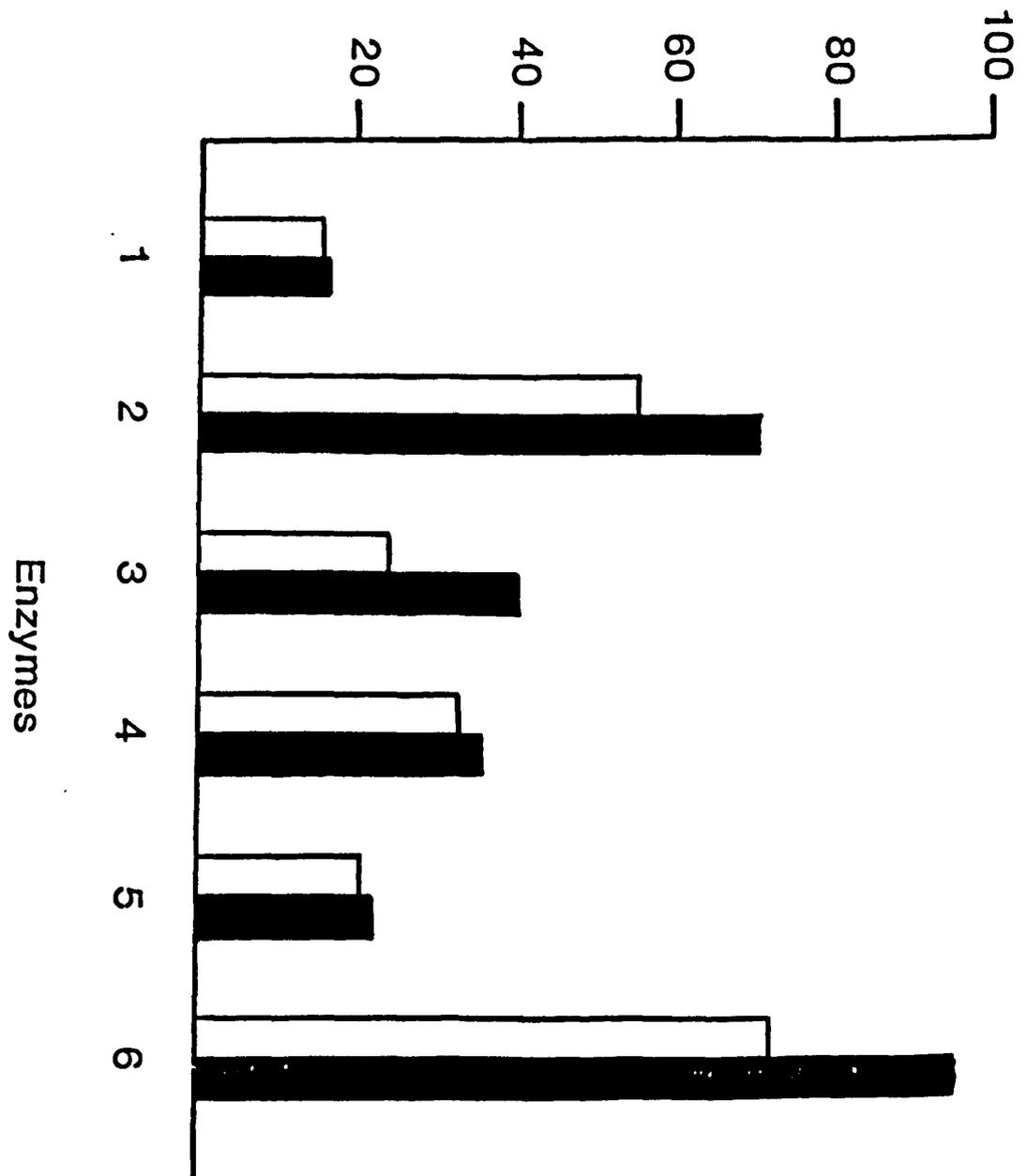


TABLE 62. Statistical analysis of the effect of enzymes on P. elegans and A. marina secretions. One-way analyses of variance comparing the effect of the six enzymes on secretions of P. elegans and A. marina. Each anovar has six levels - the six enzymes, and each level containing two readings (i.e. data from the two experiments). The analyses were conducted on the arcsine values in Appendix III, Table 15, p. 531.

| Species | Enzymes | Factors | Sum of squares (Ss) | Mean squares (Ms) | D.F | F-ratio | Probability |
|------------------------------------|-------------------|---------|---------------------|-------------------|-----|---------|-------------------|
| <u>Pygospio elegans</u> secretions | α -amylase | Main | 1290.8 | 258.2 | 5 | 104.5 | P < 0.001 |
| | Hyaluronidase | | | | | | |
| | Lipase | Error | 14.82 | 2.470 | 6 | | |
| | Lysozyme | Total | 1305.7 | | 11 | | ***** |
| | Pepsin | | | | | | |
| | Trypsin | | | | | | |
| <u>Arenicola marina</u> secretions | α -amylase | Main | 2772.7 | 554.6 | 5 | 12.28 | 0.005 > P > 0.001 |
| | Hyaluronidase | | | | | | |
| | Lipase | Error | 270.9 | 45.15 | 6 | | |
| | Lysozyme | Total | 3043.7 | | 11 | | **** |
| | Pepsin | | | | | | |
| | Trypsin | | | | | | |

TABLE 63. Two-way analysis of variance on the effects of enzymic digestion on secretions of *P. elegans* and *A. marina*

Factor A: two types of secretions;

Factor B: six enzymes.

| Factors | Sum of squares | Mean squares | D.F | F-ratio | Probability |
|-------------|----------------|--------------|-----|---------|--------------------|
| A | 33.82 | 33.82 | 1 | 23.29 | |
| B | 613.7 | 122.7 | 5 | 84.53 | |
| Interaction | 482.6 | 96.53 | 5 | 66.48 | P < 0.001 ***** |
| Error | 17.42 | 1.452 | 12 | | |
| Total | 1147.6 | | 23 | | |

3. Ranking of enzymes and species

The mean per cent weights (Appendix III, Tables 13 and 14, pp. 529, 530) were ranked firstly by the six enzymes for each species in turn (Table 64) and then by the four species for each enzyme (Table 65). The ranking by enzymes showed that α -amylase was the most effective, followed by pepsin, lipase, lysozyme, trypsin, and hyaluronidase in that order (sum of ranks - Table 64). The differences in enzymes between the species have already been referred to (see pp. 355-367).

The ranking by species showed that overall N. diversicolor and C. volutator secretions were the least resistant, followed by P. elegans. The most resistant secretions were those of A. marina.

A series of one-way anovars on the arcsine of the weights comparing differences between species and differences between enzymes showed the above statements were statistically significant. These anovars are not presented in the thesis.

TABLE 64. Ranking data, to show the effect of each enzyme on the secretions from the different species from Langbank (Corophium volutator and Nereis diversicolor) and Ardmore (Pygospio elegans and Arenicola marina).

| Species | α -Amylase | Hyaluronidase | Lipase | Lysozyme | Pepsin | Trypsin |
|------------------------|-------------------|---------------|--------|----------|--------|---------|
| <u>C. volutator</u> | 1= | 3 | 6 | 5 | 4 | 1= |
| <u>N. diversicolor</u> | 1 | 6 | 4 | 3 | 2 | 5 |
| <u>P. elegans</u> | 2 | 6 | 1 | 3 | 4 | 5 |
| <u>A. marina</u> | 1 | 5 | 3 | 4 | 2 | 6 |
| Sum of ranks | 5 | 20 | 14 | 15 | 12 | 17 |

TABLE 65. Ranking data, to show a comparison of all enzymes on the animal secretions from Langbank (Corophium volutator and Nereis diversicolor) and Ardmore (Pygospio elegans and Arenicola marina).

| Enzymes | <u>C. volutator</u> | <u>N. diversicolor</u> | <u>P. elegans</u> | <u>A. marina</u> |
|-------------------|---------------------|------------------------|-------------------|------------------|
| α -amylase | 1 | 2 | 3 | 4 |
| Hyaluronidase | 1 | 2 | 3 | 4 |
| Lipase | 3 | 2 | 1 | 4 |
| Lysozyme | 2 | 1 | 3 | 4 |
| Pepsin | 2 | 1 | 3 | 4 |
| Trypsin | 1 | 2 | 3 | 4 |
| Sum of ranks | 10 | 10 | 16 | 24 |

(III) How enzymic digestion alters the effect that secretions have on sedimentation

The results of this section are divided into two parts: presentation of results (1), and statistical analysis (2).

1. Presentation of results

The means ~~and standard deviations~~ of the suspended weights in the sedimentation experiments are plotted in Figures 49, 50, 51 and 52, and the original data given in Appendix III, Tables 15, 16, 17 and 18. The suspended weights decrease as the interval time increases - as expected. The highest and lowest suspended weights were obtained from the top control (sediment with secretions but no enzymic treatment) and the bottom control (sediment without secretions and no enzymic treatment). The phrases top and bottom controls will be used from now on. The suspended weights of the enzymatically treated sediments were intermediate.

The suspended weights obtained after enzymic treatment depend on the degree or effectiveness of the enzyme in digesting the secretions. The most effective enzyme produced a suspended weight that was close to the bottom control and the least effective enzyme one that was close to the top control.

With C. volutator secretions, the most effective enzymes were α -amylase and trypsin, followed by hyaluronidase, pepsin and lysozyme. The least effective enzyme was lipase (Figure 49). With N. diversicolor secretions, the most effective enzyme was α -amylase, followed by pepsin, lysozyme, lipase and trypsin, while the least effective was hyaluronidase (Figure 50). With P. elegans secretions, the most effective enzyme was lipase, followed by α -amylase, lysozyme, pepsin and trypsin. Here hyaluronidase was the least effective enzyme (Figure 51). With A. marina secretions, the most effective enzyme was

α -amylase followed by pepsin, lipase, lysozyme and hyaluronidase, and the less effective was trypsin (Figure 52).

The effects produced by the different enzymes on sedimentation were exactly the same as their effects on animal secretions (Part II of the present section p. 355). . The more effective an enzyme was at digesting secretions, the faster that sediment sedimented. For example, the ranking orders of the different enzymes on N. diversicolor secretion were: (1) α -amylase, (2) pepsin, (3) lysozyme, (4) lipase, (5) trypsin and (6) hyaluronidase (Table 64, p. 369). These results were exactly the same as obtained in the present sedimentation experiment with N. diversicolor secretions (Figure 50).

Figure 49

Enzymic digestion and sedimentation of Rockware sediment containing C. volutator secretions. The suspended weights of two control sediments, with and without secretions are also shown.

Points represent means. Standard deviations were too small to plot.

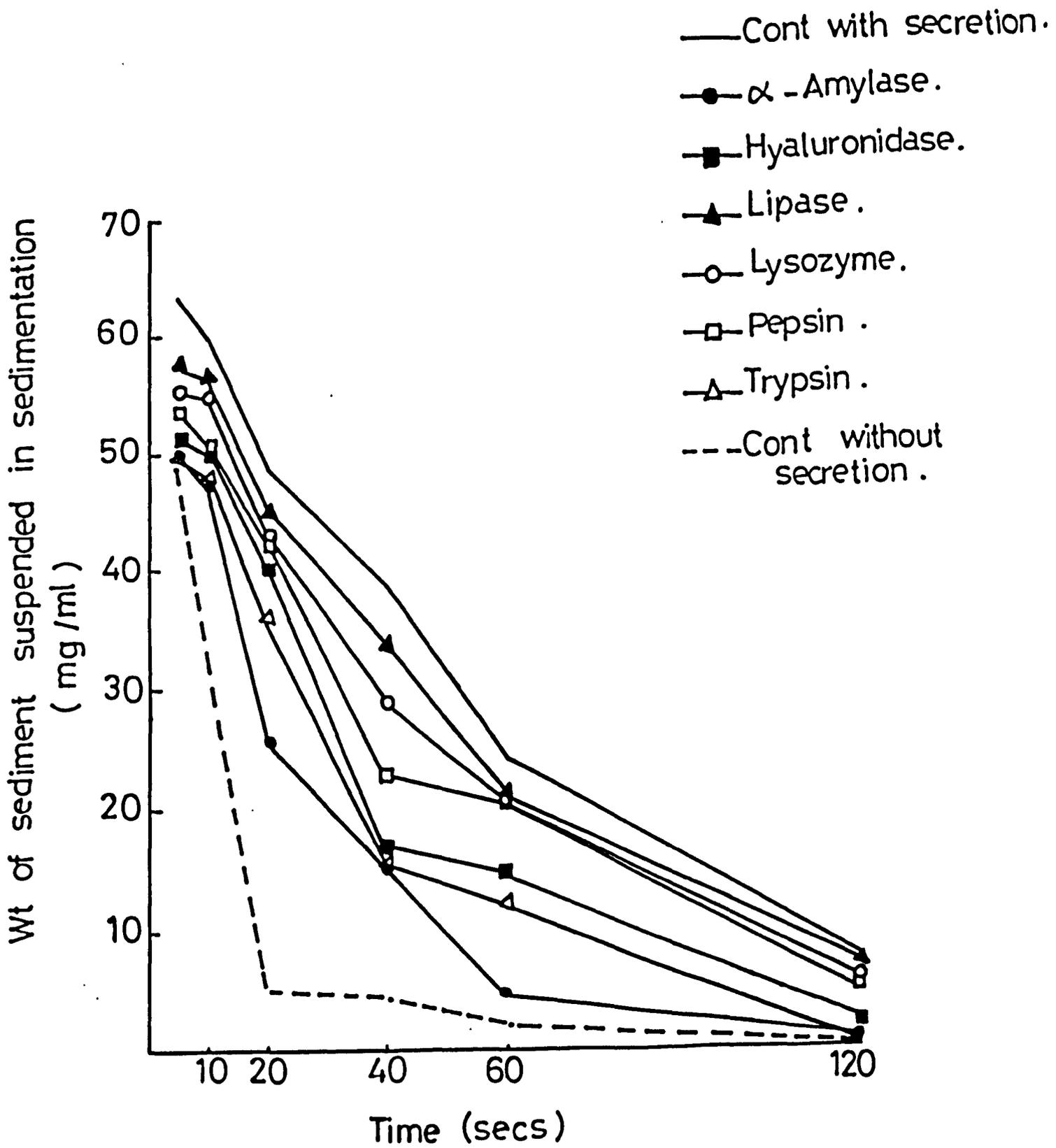


Figure 50

Enzymic digestion and sedimentation of Rockware sediment containing N. diversicolor secretions. The suspended weights of two control sediments, with and without secretions are also shown.

Points represent means. Standard deviations were too small to plot.

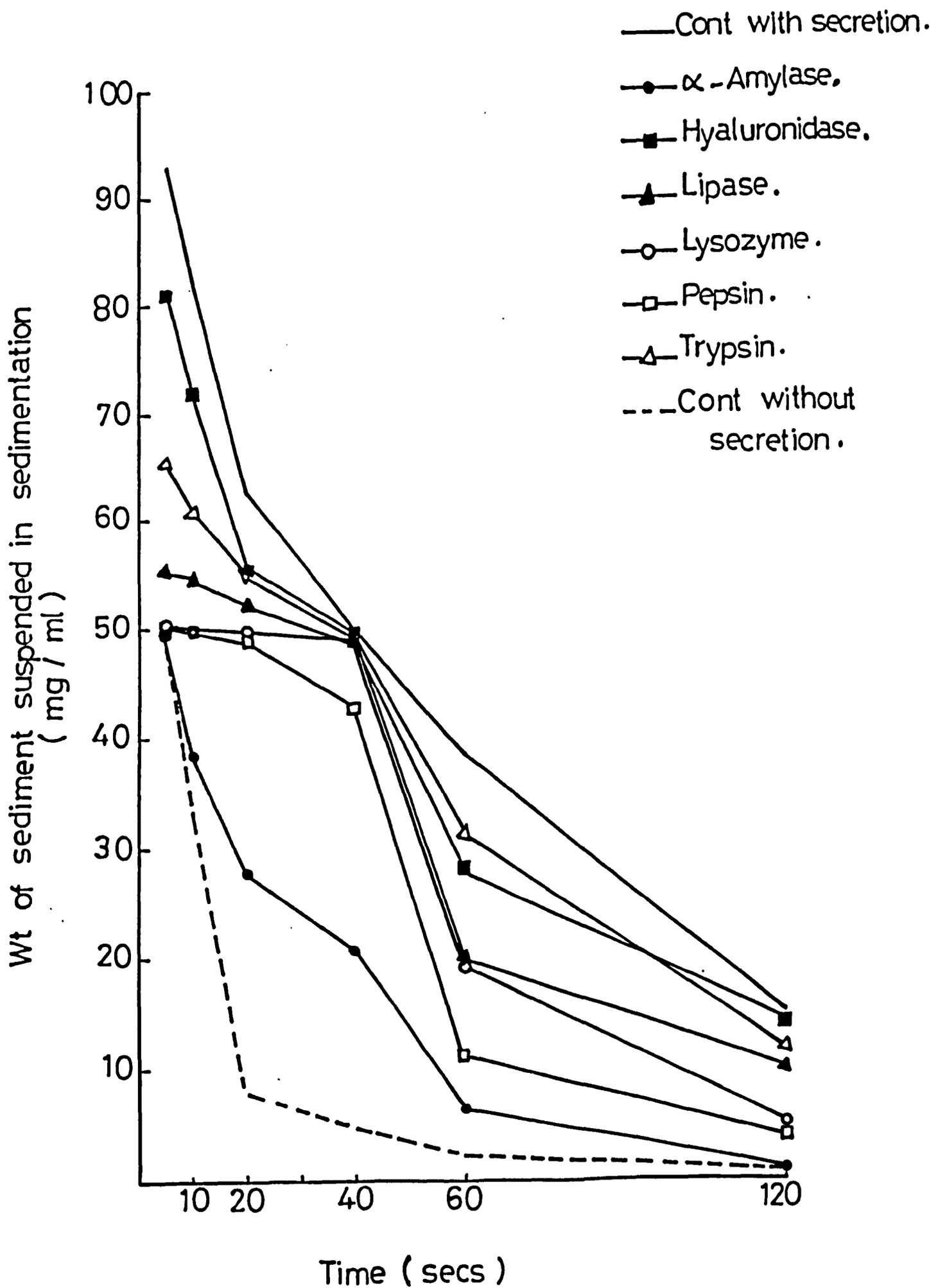


Figure 51

Enzymic digestion and sedimentation of Rockware sediment containing P. elegans secretions. The suspended weights of two control sediments, with and without secretions are also shown.

Points represent means. Standard deviations were too small to plot.

Figure 52

Enzymic digestion and sedimentation of Rockware sediment containing A. marina secretions. The suspended weights of two control sediments, with and without secretions are also shown.

Points represent means. Standard deviations were too small to plot.

2. Statistical analysis

Since the suspended weights obtained in the present sedimentation results are found to be very much related to the effect of the different enzymes obtained in the previous part in the present section (Part II, p. 355), I, therefore, decided to compare the results obtained from both parts (i.e. Parts II and III).

The data were analysed using a series of linear regressions. This series contained twenty-four regressions - one regression for each of the six time intervals for each of the four species. Each regression compared the weights remaining after enzymic digestion (x) with the suspended weights obtained during sedimentation (y). The regressions are shown in Figures 53-56 and their equations, correlation coefficients (R), and t values in Tables 66-69. The results were significant in eighteen out of the twenty-four analyses. This means that in general, there is a significant relationship between the weights of secretions remaining after enzymic digestion and the equivalent suspended weights obtained during sedimentation.

Figure 53

Relationship between the weights remaining after enzymic digestion (mg/g) (on the x-axis), and the suspended dry weights (mg/ml) (on the y-axis) for C. volutator secretions.

The six lines represent the six time intervals:

○ 5 seconds, □ 10 seconds, △ 20 seconds, ● 40 seconds,
■ 60 seconds, and ▲ 120 seconds.

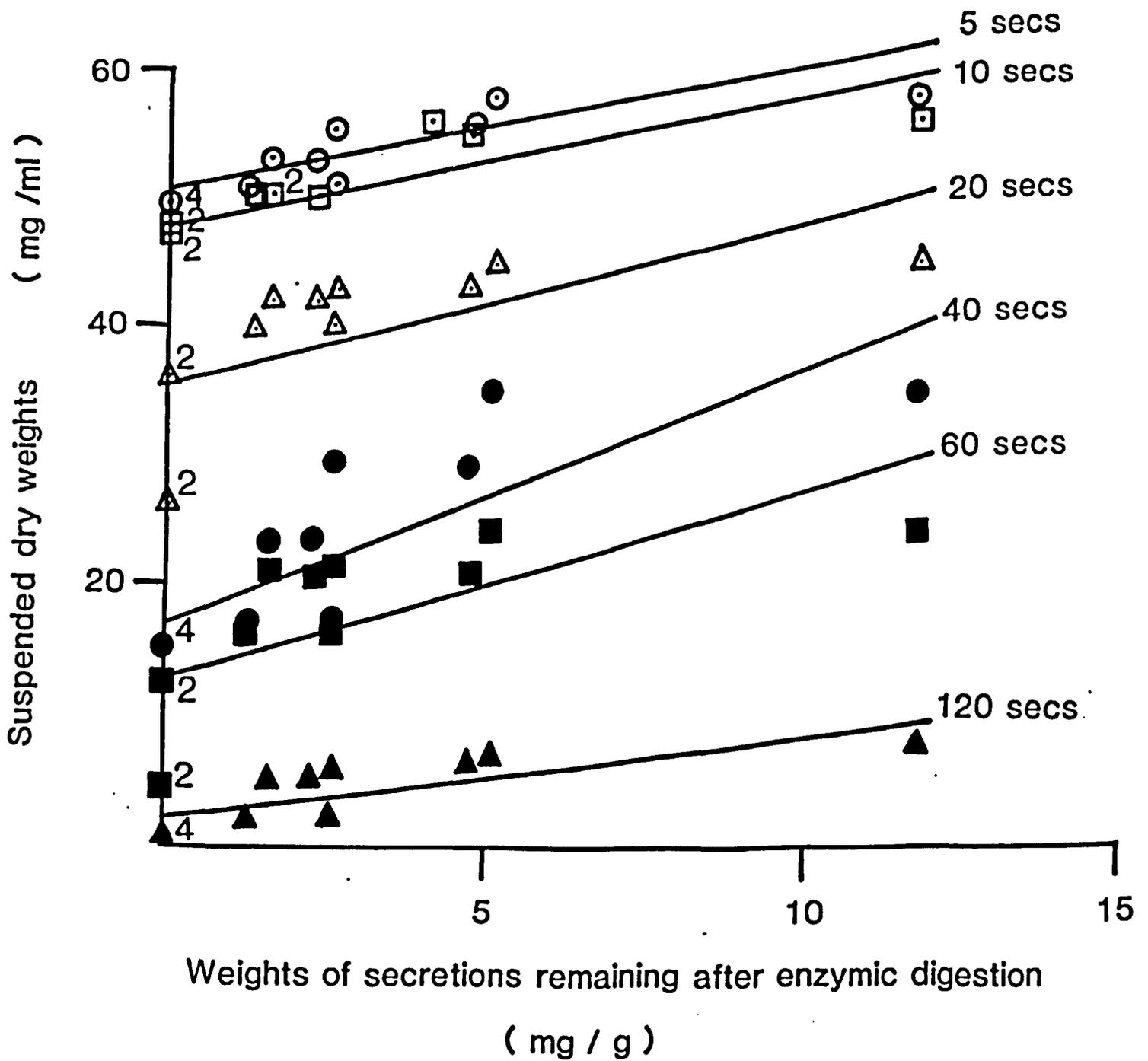


Figure 54

Relationship between the weights remaining after enzymic digestion (mg/g) (on the x-axis), and the suspended dry weights (mg/ml) (on the y-axis) for N. diversicolor secretions. The six lines represent the six time intervals: ⊙ 5 seconds, ◻ 10 seconds, △ 20 seconds, ● 40 seconds, ■ 60 seconds, and ▲ 120 seconds.

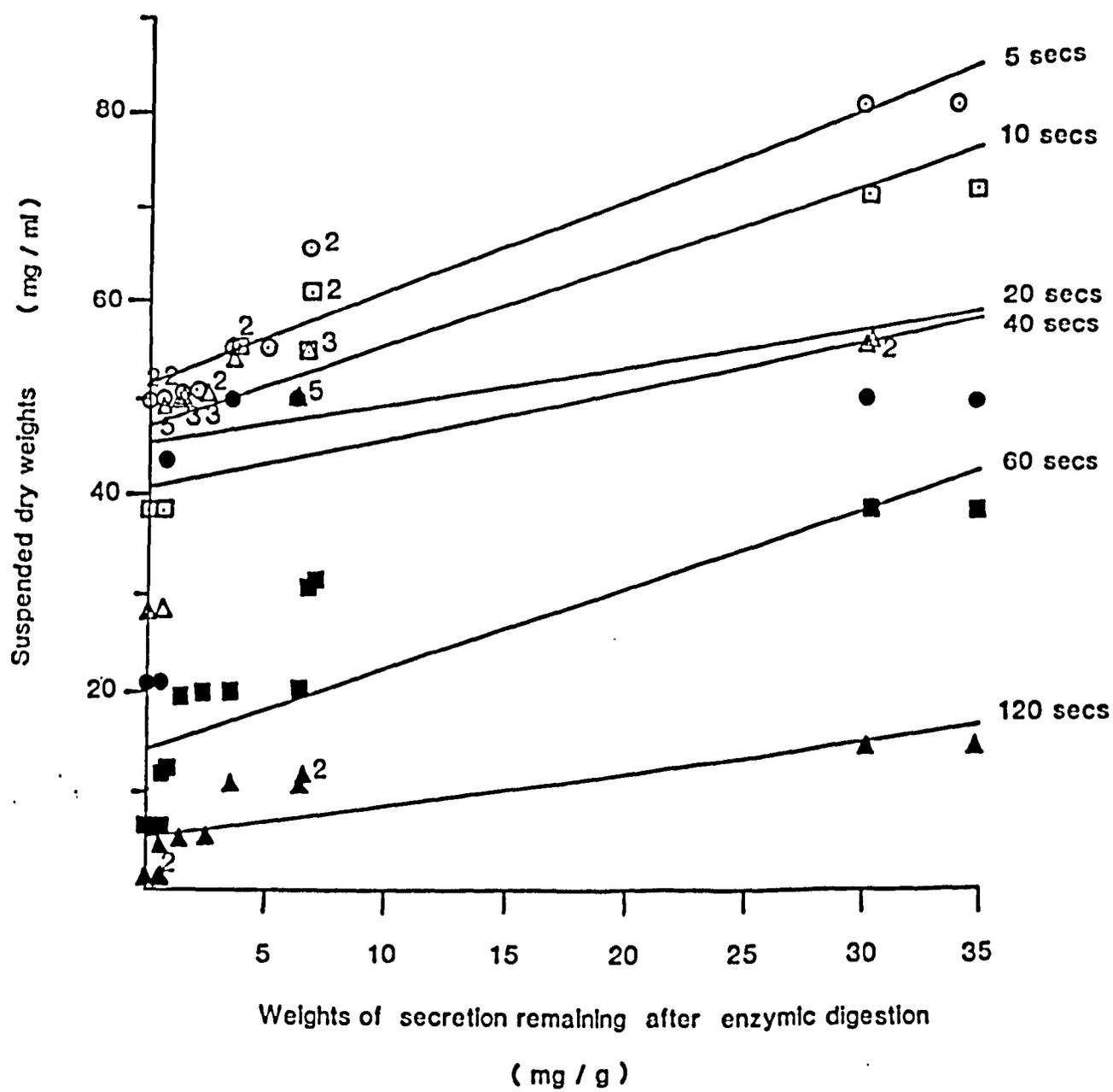


Figure 55

Relationship between the weights remaining after enzymic digestion (mg/g) (on the x-axis), and the suspended dry weights (mg/ml) (on the y-axis) for P. elegans secretions.

The six lines represent the six time intervals:

⊙ 5 seconds, ◻ 10 seconds, ▲ 20 seconds, ● 40 seconds,
■ 60 seconds, and ▲ 120 seconds.

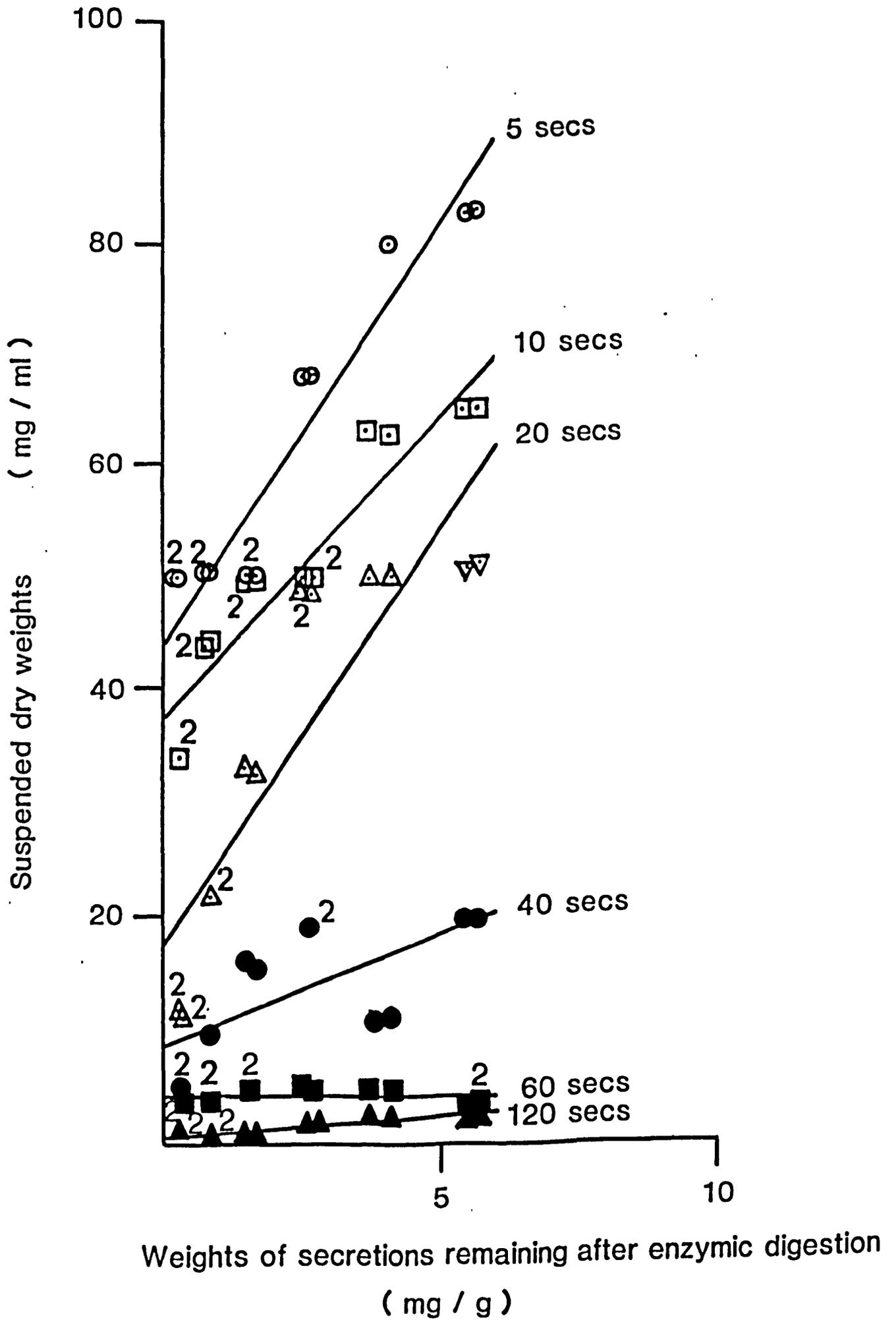


Figure 56

Relationship between the weights remaining after enzymic digestion (mg/g) (on the x-axis), and the suspended dry weights (mg/ml) (on the y-axis) for A. marina secretions.

The six lines represent the six time intervals:

⊙ 5 seconds, ◻ 10 second, △ 20 seconds, ● 40 seconds,
■ 60 seconds, and ▲ 120 seconds.

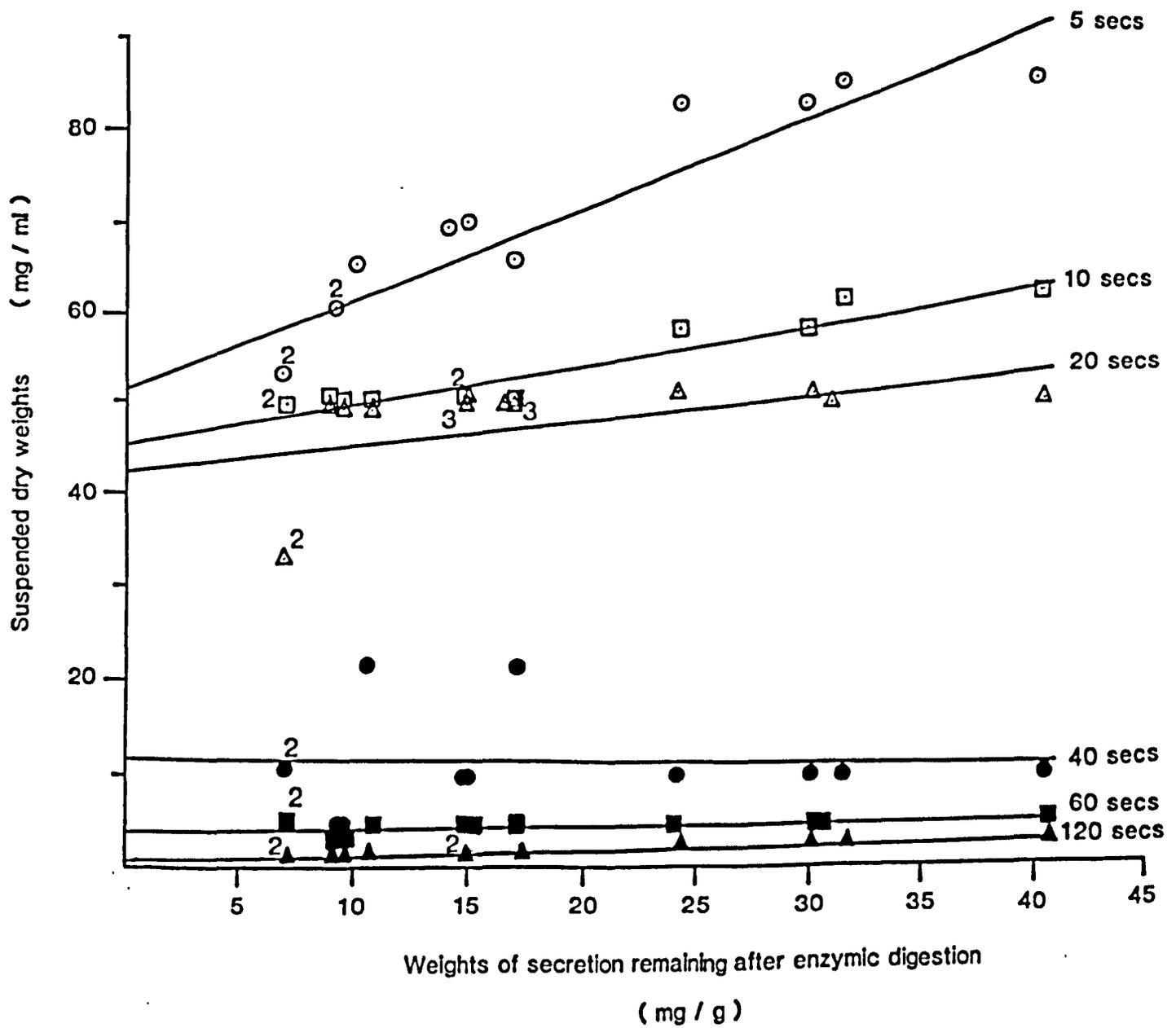


TABLE 66. Linear regression of weights remaining after enzymic treatment (mg/g) (x) against suspended weights obtained during sedimentation (mg/ml) (y) for Rockware sediment treated with Corophium volutator secretions. N = number of data pairs; R = correlation coefficient; S.E. = standard error about the regression; t = students t that slope is different from zero; probability = probability of t that slope of regression is significantly different from zero.

| Time | $y = a + bx$ | N | R | S.E. | T (B = 0) | Probability |
|-------------|-----------------------|----|--------|--------|-----------|----------------------------|
| 5 seconds | $y = 50.79 + 0.7745X$ | 12 | 0.8443 | 0.1554 | 4.983 | $P < 0.001$ ***** |
| 10 seconds | $y = 48.84 + 0.8572X$ | 12 | 0.8204 | 0.1889 | 4.538 | $P < 0.001$ ***** |
| 20 seconds | $y = 35.34 + 1.269 X$ | 12 | 0.6352 | 0.4881 | 2.600 | $0.05 > P > 0.02$ * |
| 40 seconds | $y = 17.18 + 1.943 X$ | 12 | 0.8353 | 0.4043 | 4.805 | $P < 0.001$ ***** |
| 60 seconds | $y = 12.89 + 1.430 X$ | 12 | 0.7060 | 0.4535 | 3.153 | $0.01 > P > 0.001$ **** |
| 120 seconds | $y = 2.123 + 0.6304X$ | 12 | 0.8022 | 0.1484 | 4.249 | $0.01 > P > 0.001$ **** |

TABLE 67. Linear regression of weights remaining after enzymic treatment (mg/g) (x) against suspended weights obtained during sedimentation (mg/ml) (y) for Rockware sediment treated with Nereis diversicolor secretions. N = number of data pairs; R = correlation coefficient; S.E. standard error about the regression; t = students t that slope is different from zero; probability = probability of t that slope of regression is significantly different from zero.

| Time | $y = a + bx$ | N | R | S.E. | T (B = 0) | Probability |
|-------------|-----------------------|----|--------|---------|-----------|----------------------------|
| 5 seconds | $y = 51.45 + 0.9398X$ | 12 | 0.9450 | 0.1029 | 9.133 | $P < 0.001$ ***** |
| 10 seconds | $y = 48.53 + 0.7741X$ | 12 | 0.8482 | 0.1529 | 5.063 | $P < 0.001$ ***** |
| 20 seconds | $y = 45.59 + 0.3821X$ | 12 | 0.4568 | 0.2353 | 1.624 | $0.2 > P > 0.1$ |
| 40 seconds | $y = 41.33 + 0.3468X$ | 12 | 0.3757 | 0.2706 | 1.282 | $0.3 > P > 0.2$ |
| 60 seconds | $y = 15.29 + 0.7890X$ | 12 | 0.8192 | 0.1747 | 4.517 | $P < 0.001$ ***** |
| 120 seconds | $y = 5.516 + 0.3208X$ | 12 | 0.7702 | 0.08402 | 3.818 | $0.01 > P > 0.001$ **** |

392.

TABLE 68. Linear regression of weights remaining after enzymic treatment (mg/g) (x) against suspended weights obtained during sedimentation (mg/ml) (y) for Rockware sediment treated with Pygospio elegans secretions. N = number of data pairs; R = correlation coefficient; S.E. = standard error about the regression; t = students t that slope is different from zero; probability = probability of t that slope of regression is significantly different from zero.

| Time | $y = a + bx$ | N | R | S.E. | T (B = 0) | Probability |
|-------------|-------------------------|----|---------|---------|-----------|----------------------------|
| 5 seconds | $y = 44.97 + 7.431 X$ | 12 | 0.9532 | 0.7454 | 9.969 | $P < 0.001$ ***** |
| 10 seconds | $y = 37.27 + 5.499 X$ | 12 | 0.9430 | 0.6137 | 8.960 | $P < 0.001$ ***** |
| 20 seconds | $y = 17.25 + 7.443 X$ | 12 | 0.8897 | 1.208 | 6.161 | $P < 0.001$ ***** |
| 40 seconds | $y = 8.563 + 1.972 X$ | 12 | 0.6922 | 0.6503 | 3.033 | $0.01 > P > 0.001$ **** |
| 60 seconds | $y = 4.373 - 0.07742X$ | 12 | -0.1986 | 0.1208 | -0.6403 | $0.6 > P > 0.5$ |
| 120 seconds | $y = 0.5828 + 0.3515 X$ | 12 | 0.9098 | 0.05072 | 6.930 | $P < 0.001$ ***** |

TABLE 69. Linear regression of weights remaining after enzymic treatment (mg/g) (x) against suspended weights obtained during sedimentation (mg/ml) (y) for Rockware sediment treated with Arenicola marina secretions. N = number of data pairs; R = correlation coefficient; S.E. = standard error about the regression; t = students t that slope is different from zero; probability = probability of t that slope of regression is significantly different from zero.

| Time | $y = a + bx$ | N | R | S.E. | T (B = 0) | Probability |
|-------------|-------------------------|----|----------|----------|-----------|----------------------|
| 5 seconds | $y = 50.80 + 1.021 X$ | 12 | 0.9314 | 0.1262 | 8.092 | $P < 0.001$ ***** |
| 10 seconds | $y = 45.88 + 0.4011 X$ | 12 | 0.9428 | 0.04486 | 8.942 | $P < 0.001$ ***** |
| 20 seconds | $y = 42.00 + 0.2820 X$ | 12 | 0.5000 | 0.1544 | 1.826 | $0.1 > P > 0.05$ |
| 40 seconds | $y = 11.48 - 0.02411X$ | 12 | -0.05062 | 0.1505 | -0.1603 | $0.9 > P > 0.8$ |
| 60 seconds | $y = 3.957 + 0.01829X$ | 12 | 0.3093 | 0.01778 | 1.029 | $0.4 > P > 0.3$ |
| 120 seconds | $y = 0.8162 + 0.05389X$ | 12 | 0.9396 | 0.006206 | 8.684 | $P < 0.001$ ***** |

(IV) Refinement of the sedimentation procedureMaterials and Methods

In the animal secretion experiment the concentration of sediment used was ≈ 43 g dry sediment/500 ml $\doteq 86$ mg/ml. The five-second sedimentation readings in the secretion experiments were significantly higher than this (Figures 42, 43, 44 and 45). The reason for this apparent anomaly was not immediately obvious, since the cylinders were well mixed before the beginning of the experiments. I therefore designed an experiment in which I took samples at different depths to determine the distribution of weights and particle sizes down the column at successive time intervals.

The experiment was conducted with Rockware sediment and the secretions of C. volutator and N. diversicolor exactly as previously except that samples were taken at 5, 10 and 20 cm depth. A control sediment with no secretions was also run. Four replicate 2-ml samples were taken at each depth at each time. Two of the replicates were dried and weighed as previously. One of the remaining two was used for particle size measurements. The sediment in this replicate was washed in distilled water X6, ashed at 600°C for six hours, washed in distilled water X6, and dried at 60°C for 24 hours (the remaining replicate acted as a reserve).

Particle size was measured by taking the longest and shortest Feret diameter of sand grains (a Feret diameter is the distance between two tangents on opposite sides of particle (Allen, 1975, p. 131)). One hundred sand grains were selected at random from each replicate. The total number of samples (replicates) whose particle size was measured was three treatments X6 times X3 depths = 54; since 100 sand grains were measured from each sample (i.e. replicate), 54 x 100

= 5400 sand grains were measured in total.

Because of the large number, I wrote a computer programme in BASIC to calculate (i) the mean of the longest and shortest diameter of each individual sand grain, and (ii) the means and standard deviations of these diameters for the 100 sand grains from each sample. A flow diagram, a listing and a run of the programme are shown in Figures 57, 58 and 59.

Figure 57

A flow diagram of the computer programme.

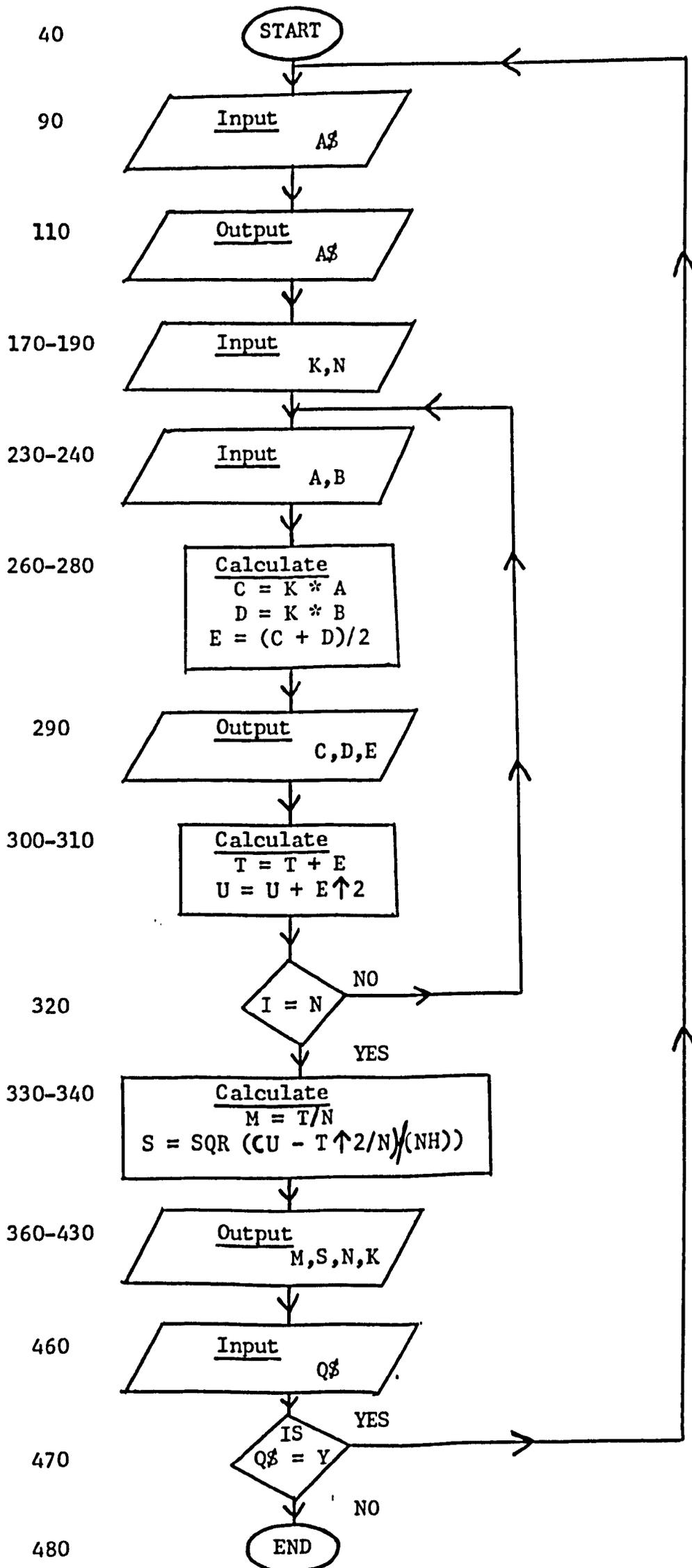


Figure 58

Listing of the computer programme.

```

10 REM ***SEDIMENT PARTICLE SIZE***B.A-HUSSAIN***AUGUST 1984***
20 PRINT "THIS PROGRAM CALCULATES THE MEANS AND STANDARD DEVIATIONS OF SEDIMENT
PARTICLES FROM THEIR LONGEST AND SHORTEST LENGTH DIA
METERS"
30 PRINT:PRINT:PRINT
40 CLEAR
50 LPRINT "THIS PROGRAM CALCULATES THE MEANS AND STANDARD DEVIATIONS OF"
60 LPRINT "THE DIAMETERS OF SEDIMENT PARTICLES FROM THE MEAN OF"
70 LPRINT "THE LONGEST AND SHORTEST DIAMETER OF EACH PARTICLE"
80 LPRINT:LPRINT
90 PRINT:PRINT:PRINT
100 INPUT "ENTER DETAILS OF SAMPLE ":A$
110 PRINT
120 LPRINT A$
130 LPRINT:LPRINT
140 LPRINT"DIMENSIONS OF INDIVIDUAL SAND GRAINS (MICROMETERS)"
150 LPRINT
160 LPRINT "LONGEST DIMENSION","SHORTEST DIMENSION","MEAN"
170 LPRINT
180 INPUT "ENTER MULTIPLICATION FACTOR THAT CONVERTS YOUR MEASUREMENT TO MICROME
TERS":K
190 PRINT
200 INPUT "ENTER THE NUMBER OF SAND GRAINS":N
210 PRINT
220 FOR I=1 TO N
230 PRINT
240 INPUT "ENTER THE LONGEST DIMENSION OF THE SAND GRAIN":A
250 INPUT "ENTER THE SHORTEST DIMENSION OF THE SAND GRAIN":B
260 PRINT:PRINT
270 C=K*A
280 D=K*B
290 E=(C+D)/2
300 LPRINT TAB(6):C:TAB(35):D:TAB(55):E
310 T=T+E
320 U=U+E^2
330 NEXT I
340 M=T/N
350 S=SQR((U-T^2/N)/(N-1))
360 LPRINT:LPRINT
370 LPRINT "MEAN PARTICLE SIZE (MICROMETERS)=":M
380 LPRINT "STANDARD DEVIATION (MICROMETERS)=":S
390 LPRINT "NUMBER OF SAND GRAINS MEASURED ":N
400 LPRINT:LPRINT"MULTIPLICATION FACTOR (MICROMETERS)=":K
410 PRINT "MEAN PARTICLE SIZE (MICROMETERS)=":M
420 PRINT "STANDARD DEVIATION (MICROMETERS)=":S
430 PRINT "NUMBER OF SAND GRAINS MEASURED ":N
440 PRINT:PRINT"MULTIPLICATION FACTOR (MICROMETERS)=":K
450 PRINT:PRINT:PRINT
460 LPRINT:LPRINT:LPRINT:LPRINT:LPRINT:LPRINT:LPRINT:LPRINT:LPRINT:LPRINT
470 INPUT "PRESS Y TO CONTINUE":Q$
480 IF Q$="Y"THEN 40
490 END

```

Figure 59

A run of the programme with one example: N. diversicolor
at 10 cm depth at 5 seconds.

THIS PROGRAM CALCULATES THE MEANS AND STANDARD DEVIATIONS OF
THE DIAMETERS OF SEDIMENT PARTICLES FROM THE MEAN OF
THE LONGEST AND SHORTEST DIAMETER OF EACH PARTICLE

N.D/10CM/5SEC

DIMENSIONS OF INDIVIDUAL SAND GRAINS (MICROMETERS)

| LONGEST DIMENSION | SHORTEST DIMENSION | MEAN |
|-------------------|--------------------|--------|
| 278.52 | 240.54 | 259.53 |
| 455.76 | 240.54 | 348.15 |
| 291.18 | 240.54 | 265.86 |
| 291.18 | 278.52 | 284.85 |
| 354.48 | 265.86 | 310.17 |
| 354.48 | 202.56 | 278.52 |
| 367.14 | 227.88 | 297.51 |
| 329.16 | 253.2 | 291.18 |
| 367.14 | 240.54 | 303.84 |
| 316.5 | 240.54 | 278.52 |
| 405.12 | 316.5 | 360.81 |
| 354.48 | 227.88 | 291.18 |
| 367.14 | 240.54 | 303.84 |
| 329.16 | 265.86 | 297.51 |
| 329.16 | 202.56 | 265.86 |
| 455.76 | 240.54 | 348.15 |
| 329.16 | 253.2 | 291.18 |
| 392.46 | 253.2 | 322.83 |
| 430.44 | 227.88 | 329.16 |
| 316.5 | 240.54 | 278.52 |
| 303.84 | 291.18 | 297.51 |
| 405.12 | 265.86 | 335.49 |
| 316.5 | 240.54 | 278.52 |
| 417.78 | 265.86 | 341.82 |
| 316.5 | 227.88 | 272.19 |
| 316.5 | 240.54 | 278.52 |
| 379.8 | 215.22 | 297.51 |
| 367.14 | 240.54 | 303.84 |
| 291.18 | 265.86 | 278.52 |
| 278.52 | 253.2 | 265.86 |
| 278.52 | 189.9 | 234.21 |
| 316.5 | 278.52 | 297.51 |
| 392.46 | 227.88 | 310.17 |
| 341.82 | 253.2 | 297.51 |
| 291.18 | 227.88 | 259.53 |
| 303.84 | 291.18 | 297.51 |
| 341.82 | 265.86 | 303.84 |
| 316.5 | 177.24 | 246.87 |
| 379.8 | 253.2 | 316.5 |
| 316.5 | 253.2 | 284.85 |
| 367.14 | 227.88 | 297.51 |
| 329.16 | 253.2 | 291.18 |
| 379.8 | 303.84 | 341.82 |
| 379.8 | 240.54 | 310.17 |
| 379.8 | 253.2 | 316.5 |
| 316.5 | 253.2 | 284.85 |
| 303.84 | 189.9 | 246.87 |
| 354.48 | 253.2 | 303.84 |
| 341.82 | 240.54 | 291.18 |
| 379.8 | 265.86 | 322.83 |
| 291.18 | 215.22 | 253.2 |
| 455.76 | 253.2 | 354.48 |
| 443.1 | 303.84 | 373.47 |
| 392.46 | 253.2 | 322.83 |
| 329.16 | 253.2 | 291.18 |
| 392.46 | 253.2 | 322.83 |
| 341.82 | 227.88 | 284.85 |
| 329.16 | 227.88 | 278.52 |
| 367.14 | 227.88 | 297.51 |
| 253.2 | 253.2 | 253.2 |
| 405.12 | 253.2 | 329.16 |
| 303.84 | 253.2 | 278.52 |
| 278.52 | 240.54 | 259.53 |
| 329.16 | 278.52 | 303.84 |
| 379.8 | 303.84 | 341.82 |
| 316.5 | 291.18 | 303.84 |
| 265.86 | 240.54 | 253.2 |
| 291.18 | 240.54 | 265.86 |
| 316.5 | 215.22 | 265.86 |
| 379.8 | 227.88 | 303.84 |
| 341.82 | 215.22 | 278.52 |
| 329.16 | 215.22 | 272.19 |
| 392.46 | 253.2 | 322.83 |
| 316.5 | 240.54 | 278.52 |
| 329.16 | 227.88 | 278.52 |
| 303.84 | 227.88 | 265.86 |
| 316.5 | 240.54 | 278.52 |
| 367.14 | 253.2 | 310.17 |
| 392.46 | 265.86 | 329.16 |
| 379.8 | 189.9 | 284.85 |
| 367.14 | 291.18 | 329.16 |
| 329.16 | 303.84 | 316.5 |
| 278.52 | 240.54 | 259.53 |
| 278.52 | 253.2 | 265.86 |
| 341.82 | 215.22 | 278.52 |
| 354.48 | 202.56 | 278.52 |
| 341.82 | 240.54 | 291.18 |
| 354.48 | 240.54 | 297.51 |
| 367.14 | 227.88 | 297.51 |
| 291.18 | 240.54 | 265.86 |
| 354.48 | 240.54 | 297.51 |
| 303.84 | 215.22 | 259.53 |
| 341.82 | 240.54 | 291.18 |
| 341.82 | 253.2 | 297.51 |
| 405.12 | 278.52 | 341.82 |
| 367.14 | 177.24 | 272.19 |
| 354.48 | 278.52 | 316.5 |
| 341.82 | 278.52 | 310.17 |
| 291.18 | 253.2 | 272.19 |
| 316.5 | 265.86 | 291.18 |

MEAN PARTICLE SIZE (MICROMETERS) = 294.788
STANDARD DEVIATION (MICROMETERS) = 27.6186
NUMBER OF SAND GRAINS MEASURED = 100

MULTIPLICATION FACTOR (MICROMETERS) = 126.6

Results

This section is divided into two parts: (1) weights of suspended particles; (2) sizes of suspended particles. Statistical analyses are included in each part.

1. Weights of suspended particles

The means and standard deviations of the suspended weights obtained at the different depths and for the successive time intervals are shown in Appendix III, Table 19, p. 535. These data are plotted in Figures 60, 61 and 62. There are obvious differences between the depths and the treatments. The time differences are to be expected. The differences between depths and between treatments are statistically analysed below.

Statistical analysis of results

(a) Comparison between depths

The differences between the different depths at the successive time intervals were tested statistically by analyses of variance.

For each treatment (N. diversicolor secretions, C. volutator secretions, control), a two-way analysis of variance was conducted testing depths (5, 10 and 20 cm) against time (5, 10, 20, 40, 60 and 120 seconds). These three two-way analyses of variance showed first order interactions ($P < 0.001$) and therefore nothing can be said about the main effects of depths and time (Appendix III, Table 20, p. 536).

Differences between depths at each time were therefore analysed by six 1 x 3 one-way analyses of variance for each treatment, giving eighteen 1 x 3 one-way analyses. The three levels in each one-way anovar are the three depths. These anovars are shown in Appendix III, Tables 21, 22 and 23 (pp. 537, 538, 539) respectively.

Figure 60

Means and standard deviations of suspended dry weight to show the effect of Corophium volutator secretions on sedimentation of Rockware sediment particles at three different depths (5, 10 and 20 cm).

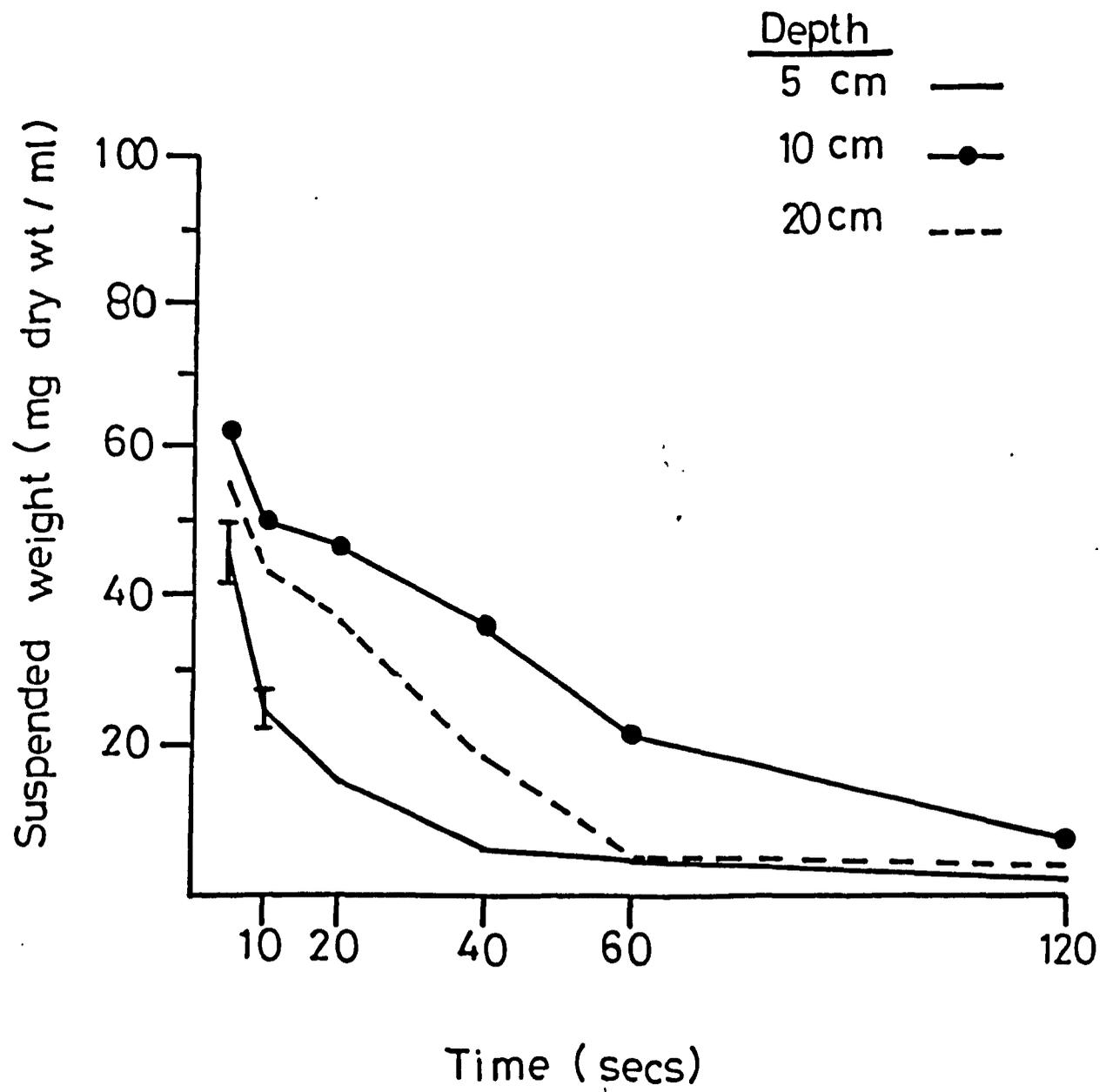


Figure 61

Means and standard deviations of suspended dry weight to show the effect of Nereis diversicolor secretions on sedimentation of Rockware sediment particles at three different depths (5, 10 and 20 cm).

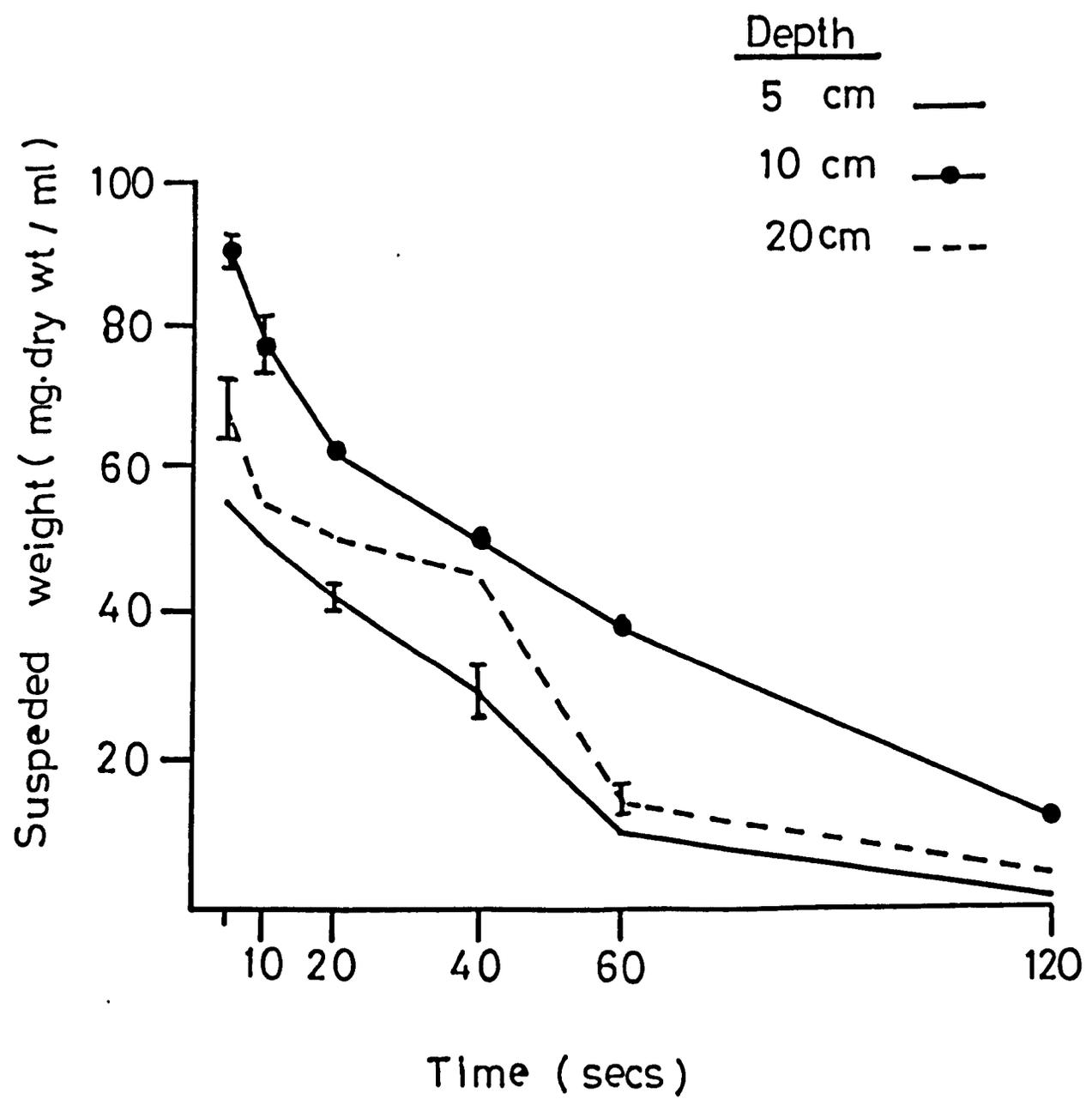
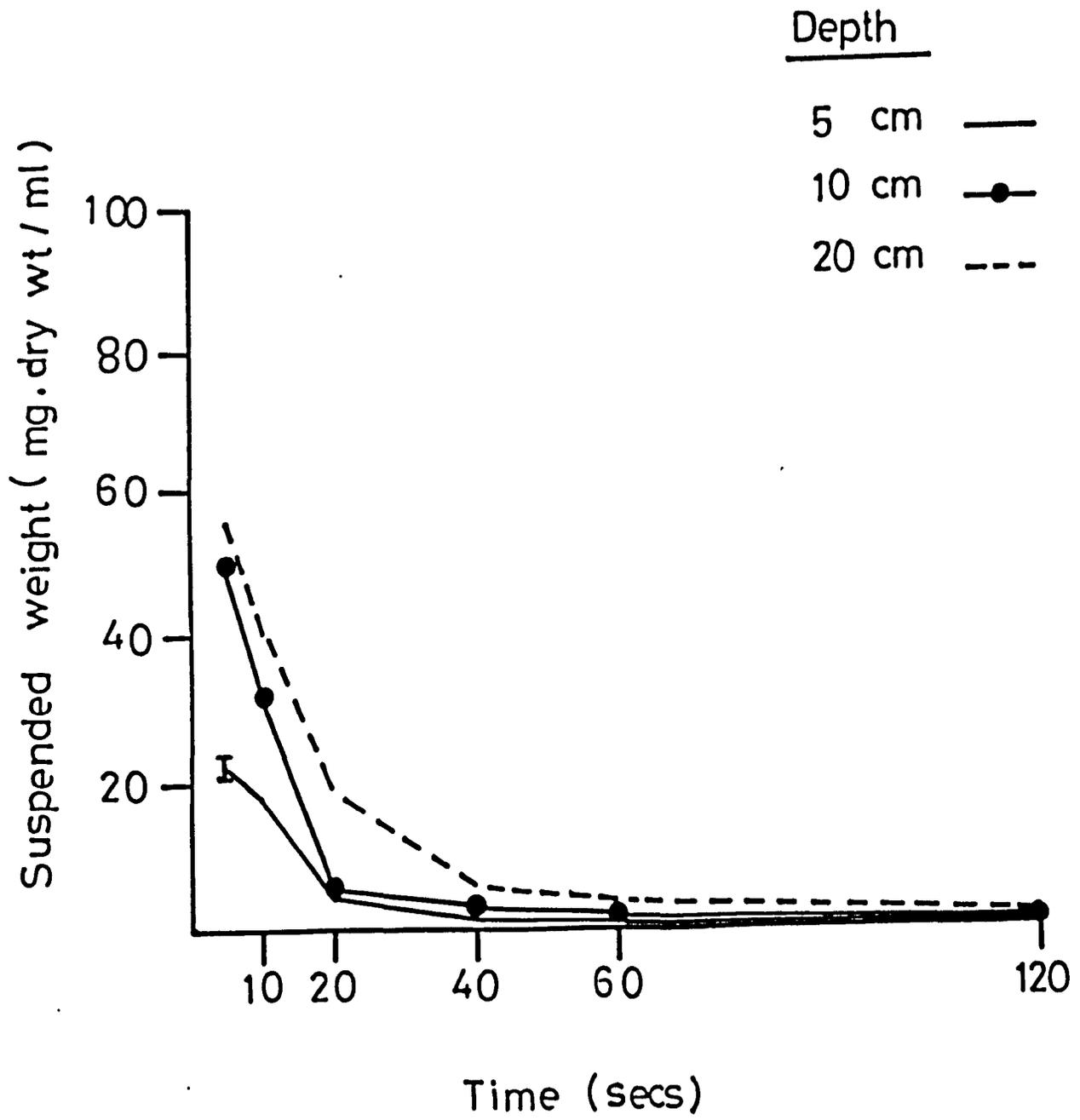


Figure 62

Means and standard deviations of suspended dry weight to show the sedimentation of the control Rockware sediment (sediment without secretions) at the three different depths (5, 10 and 20 cm).



These one-way anovars are all significant. Therefore the amount of suspended sediment at the three different depths are significantly different from each other at each time for each of the treatments. For N. diversicolor and C. volutator, the weights from the 10 cm depth were greatest, followed by those from the 20 cm and then those from the 5 cm (Appendix III, Table 19; rows 2, and 5, and Figures 60 and 61).

The results with the control sediment were different. The weights from the 20 cm depth were greatest, followed by those from the 10 cm and then those from the 5 cm (Appendix III, Table 19; rows 7, 8 and 9, and Figure 62).

(b) Comparison between treatments

The differences between the different treatments (N. diversicolor secretions, C. volutator secretions, and untreated control sediment) at the successive time intervals were also tested statistically by analyses of variance.

At each depth (5, 10 and 20 cm), a two-way analysis of variance was conducted testing treatments against time (5, 10, 20, 40, 60 and 120 seconds). These three two-way analyses of variance showed first order interactions ($P < 0.001$) and therefore nothing can be said about the main effects of treatment and time (Appendix III, Tables 24, 25 and 26, pp. 540, 541, 542).

Differences between treatments at each time were therefore analysed by six 1 x 3 one-way analyses of variance for each depth (5, 10 and 20 cm), giving eighteen 1 x 3 one-way analyses. The three levels in each one-way anovar are the three treatments. These anovars are shown in Appendix III, Tables 27, 28 and 29, pp. 543, 544, 545, respectively.

These one-way anovars are all significant except at the 20 cm depth at the 120 seconds time interval ($0.10 > P > 0.05$). Therefore the amounts of suspended sediment for the three different treatments are significantly different from each other at each depth and time (except the 120 seconds at 20 cm depth). A histogram of the data from the three graphs is presented in Figure 63 to show comparison of the three treatments at each depth to illustrate these effects.

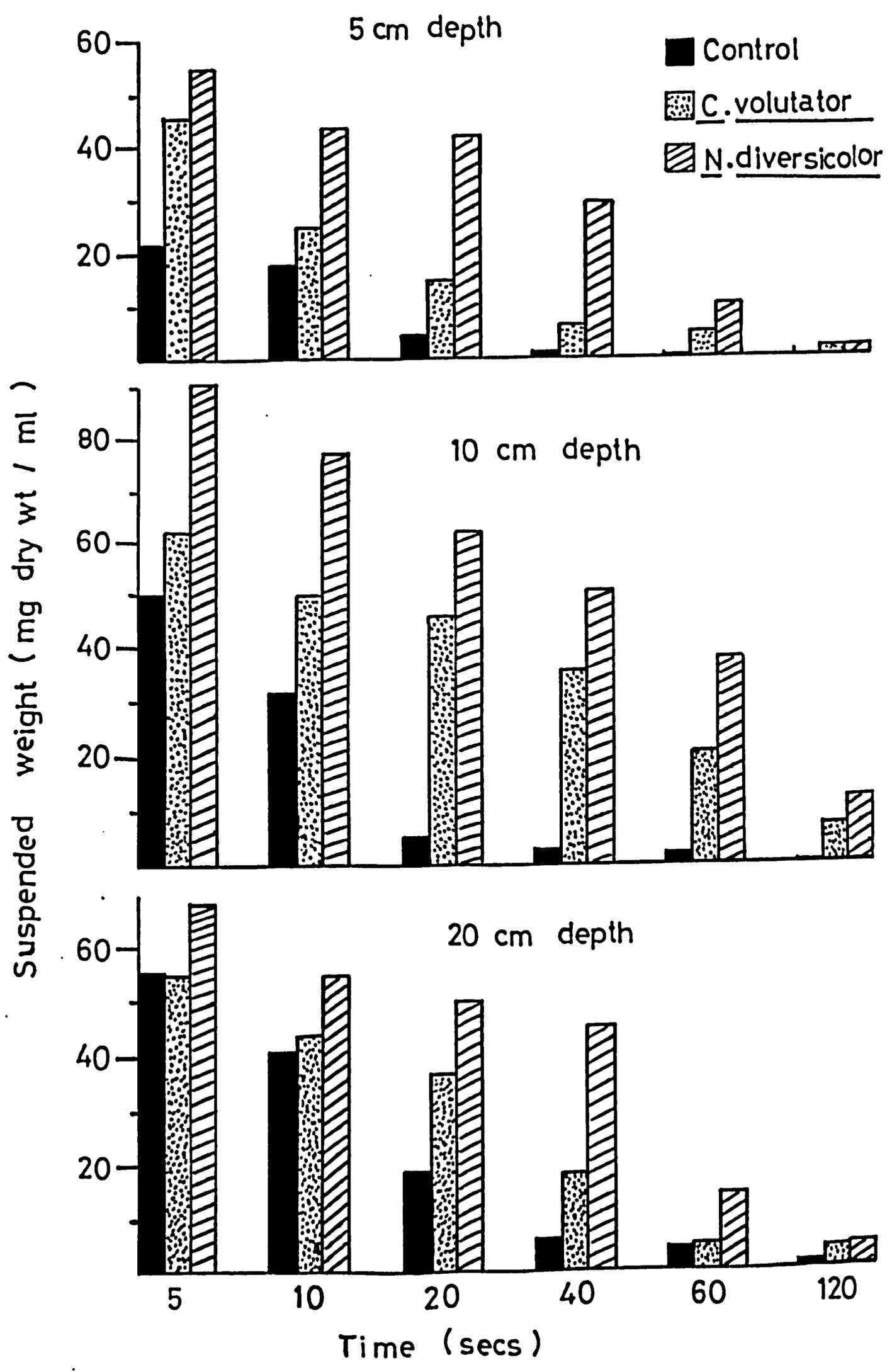
The results of these statistical tests show clearly that at each depth (5, 10 and 20 cm), the suspended weights of sediment with N. diversicolor secretions were greatest, followed by those with the C. volutator secretions, and then those of the untreated control sediment (Figure 63).

Note

The 10 cm depth samples are, of course, exactly the same as those in a previous experiment (Figures 43 (p. 325) and 45 (p. 334) and Appendix III, Table 2 (p. 518) and Appendix III, Table 4 (p. 520)).

Figure 63

Comparison of suspended weights obtained from the three different treatments (C. volutator ☒ , N. diversicolor ▨ , and the control ■) at the three different depths (5, 10 and 20 cm) during sedimentation.



2. Sizes of suspended particles

The means and standard deviations of the particle diameters obtained at the different depths for the different treatments at the successive time intervals are shown in Appendix III, Table 30, p. 546, and plotted in Figures 64 and 65.

The mean particle diameter decreased as the time interval increased for all treatments and depths. This is to be expected.

There are also obvious differences between the depths and the treatments which are statistically analysed below.

Statistical analysis of results

(a) Comparison between depths

The differences between the depths at the successive time intervals were tested statistically using student t-tests as follows.

For each treatment (N. diversicolor secretions, C. volutator secretions and the control), t-tests were conducted on eighteen comparisons (5 cm/10 cm, 5 cm/20 cm, 10 cm/20 cm, at the six times).

The eighteen t-tests for N. diversicolor are shown in Appendix III, Table 31, p. 547, for C. volutator in Appendix III, Table 32, p. 548, and for the control sediment in Appendix III, Table 33, p. 549. Significant differences were found in 16/18 Nereis comparisons, 16/18 Corophium comparisons and all the control comparisons.

For N. diversicolor and for C. volutator, the largest particle sizes were found at the 10 cm depth, followed by those from the 20 cm and then from 5 cm (Appendix III, Table 30; rows 1-6 and Figure 64). However, in the control, the largest particle sizes were found at the 20 cm depth, followed by those from the 10 cm and then from 5 cm

(Appendix III, Table 30; rows 7-9 and Figure 64).

(b) Comparison between treatments

The differences between the different treatments (N. diversicolor secretions, C. volutator secretions and the control sediment) were tested statistically using student t-tests as follows.

At each depth and time, t-tests were conducted on N. diversicolor secretions/C. volutator secretions, N. diversicolor secretions/control, and C. volutator secretions/control. The resultant fifty-four t-tests (three comparisons x six times x three depths) are shown in Appendix III, Tables 34, 35 and 36, pp. 550, 551, 552. Statistically significant differences were found in all the 5 cm and 10 cm comparisons (Appendix III, Tables 34 and 35) and in 15/18 of the 20 cm comparisons (Appendix III, Table 36, p. 552).

Therefore, at all three depths the particle sizes obtained from the N. diversicolor secretions were greater than those from the C. volutator secretions which were greater than from the controls. Figure 65 shows comparisons of the three different treatments at each depth (5, 10 and 20 cm) to illustrate these effects.

Figure 64

Means and standard deviations of particle diameter obtained at 5 cm (□), 10 cm (◻), and 20 cm (■) depths in the sedimentation cylinder for the control, and treated sediment with each of C. volutator and N. diversicolor.

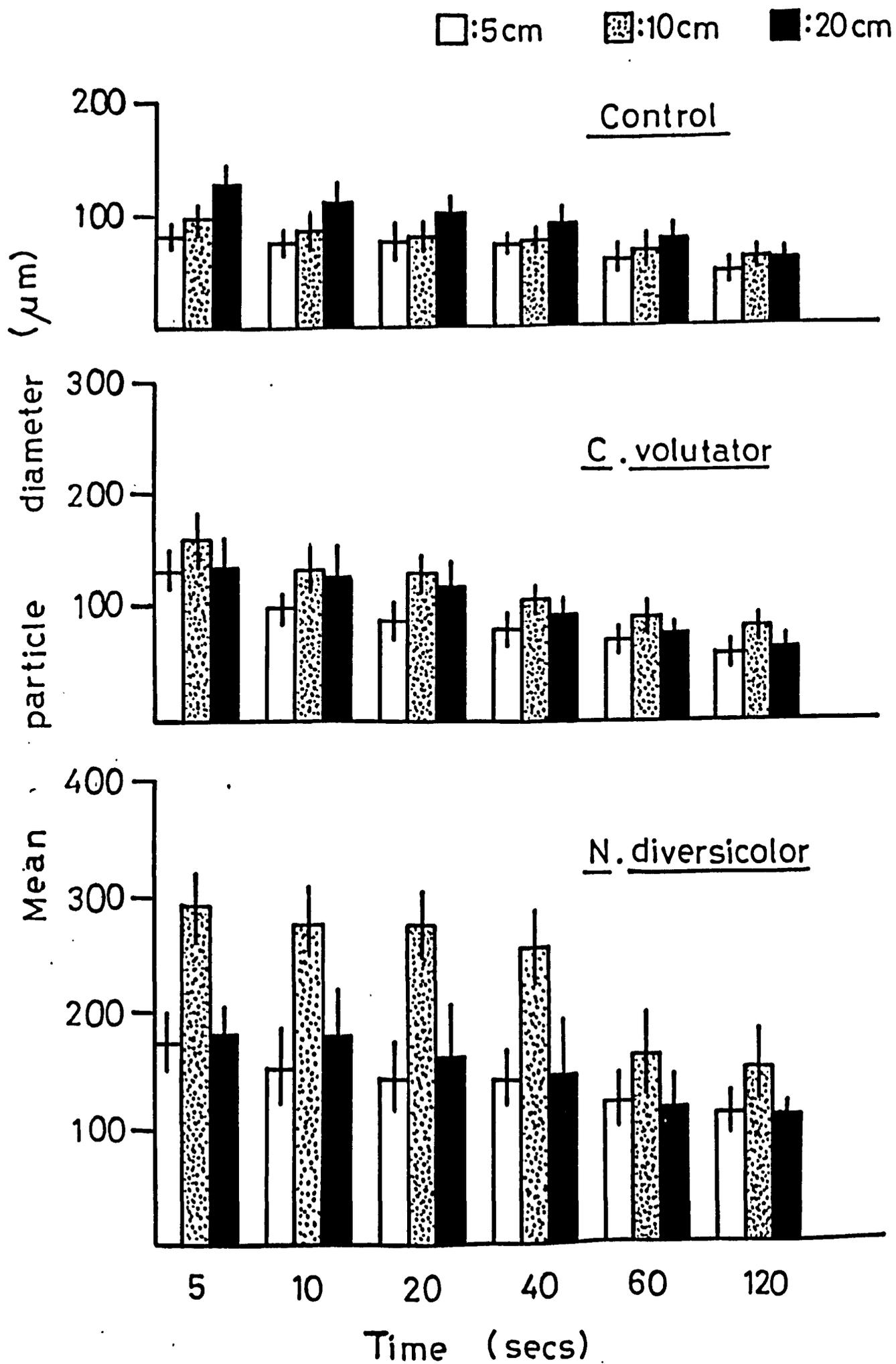
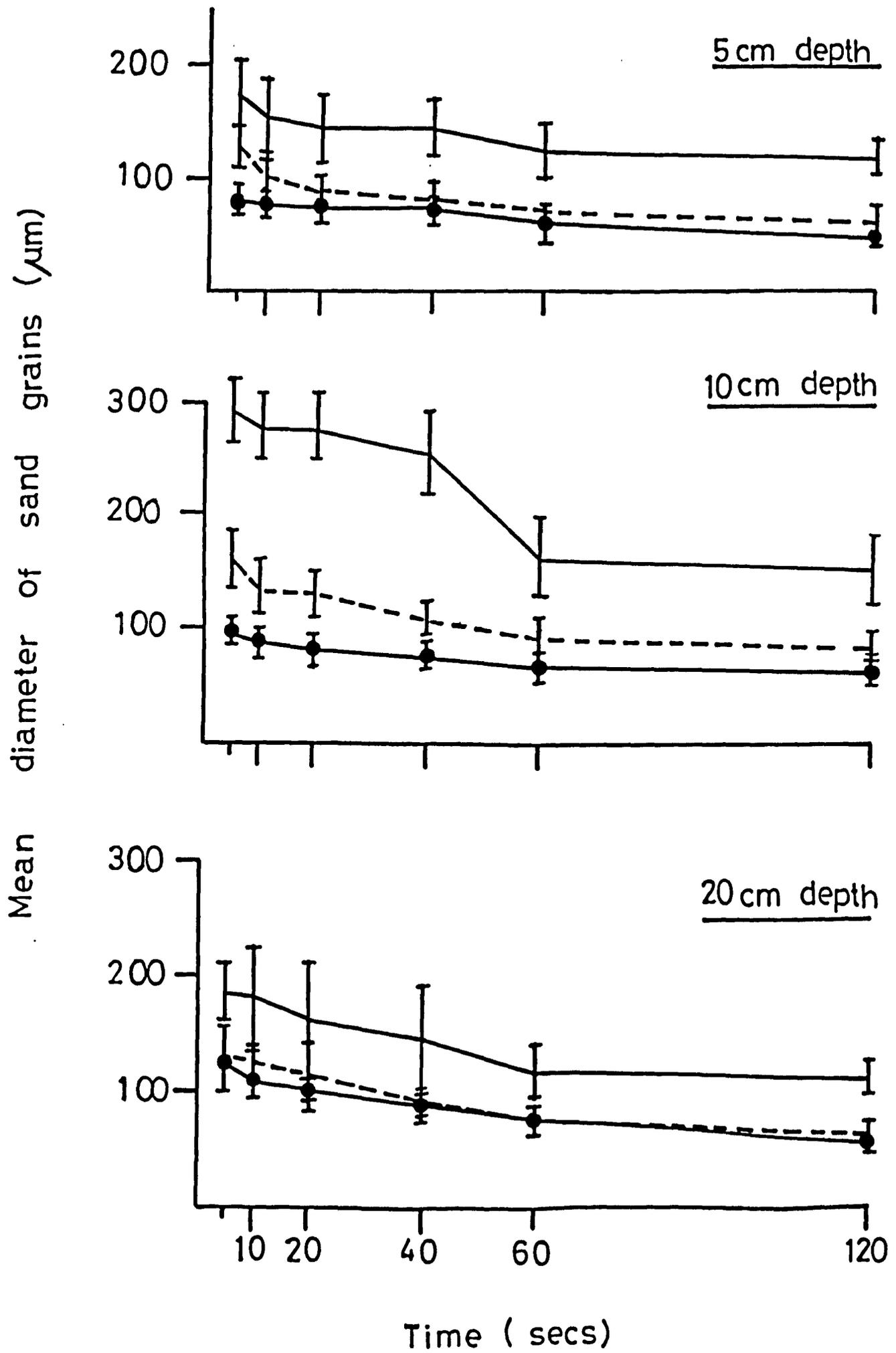


Figure 65

Means and standard deviations of the mean longest and shortest diameter of sand grains obtained at six different time intervals from the different treatments (control (●), C. volutator (----), and N. diversicolor (—)) at the different depths (5, 10 and 20 cm) in the sedimentation cylinder.



SECTION III

Discussion

The discussion in this section is divided into four parts. The first (I) discusses the effects of animal secretions on sedimentation, the second (II) discusses the effects of different enzymes on animal secretions, the third (III) explains how enzymic digestion of secretions alters the effect that secretions had on sedimentation. The fourth (IV) briefly describes the refinement of the sedimentation procedure.

I can find no references in the literature to similar work. Therefore, all my discussion will be based on my **results**. One exception to this is in part II (Table 70, p. 422) which contains a list of references showing the known substrates, and the chemical bonds broken by each enzyme.

(I) Effect of animal secretions on sedimentation

The results of this part can be summarised as follows. Secretions from benthic species at Langbank (C. volutator and N. diversicolor) and at Ardmore (P. elegans and A. marina) dramatically decreased sedimentation, except for S. armiger (Ardmore) which had no effect.

At Langbank, the greatest effect was produced by secretions of the mixed species (C. volutator and N. diversicolor), followed by N. diversicolor and C. volutator. At Ardmore, the greatest effect on sedimentation was produced by A. marina followed by P. elegans.

These effects occurred with Rockware sediment as well as with the two natural sediments, and hence are not specific to one sediment type. However, the effects were greater for the two natural sediments than for Rockware sediment. This may be caused by animals producing more secretions in their natural sediment, or by slight particle size

differences between the natural and Rockware sediments.

At the 5-second time interval, the suspended weights for C. volutator and N. diversicolor secretions in Langbank sediment were 1.8 and 2.4 times the suspended weight of the control sediment. The equivalent figures for P. elegans and A. marina secretions in Ardmore sediment were 1.9 and 2.5. This means that C. volutator and P. elegans have approximately similar effects (1.8 and 1.9) as do N. diversicolor and A. marina (2.4 and 2.5), although the similarities are probably casual.

Comparisons between the four species using a ranking system (Section III, Table 59, p. 340), showed that secretions of A. marina followed by those of N. diversicolor produced the greatest effect on sedimentation at the 5- and 10-second time intervals. However, secretions of N. diversicolor followed by secretions of C. volutator had the greatest effect at 40, 60 and 120 seconds. This may be very important in the field, since large and small particles are in suspension at the beginning of the experiment, while towards the end of the experiment only small particles remain.

The mean particle sizes at 5 and 10 seconds are $294.8 \pm 27.62 \mu\text{m}$ and $278 \pm 30.57 \mu\text{m}$, while at 40, 60 and 120 seconds the particle sizes are $257.1 \pm 36.27 \mu\text{m}$, $164.3 \pm 35.64 \mu\text{m}$, and $152.2 \pm 33.55 \mu\text{m}$ (Appendix III, Table 30; row 2, p. 546). Hence in the field, the secretions of A. marina are more likely to reduce sedimentation of resuspended particles in the size range $278 \pm 30.57 \mu\text{m}$ to $294.8 \pm 27.62 \mu\text{m}$, and so these particles will be carried further by currents than particles of a similar size without secretions. Conversely, the secretions of C. volutator are more likely to reduce sedimentation of resuspended particles in the size range $84.29 \pm 11.73 \mu\text{m}$ to $107.4 \pm 13.56 \mu\text{m}$, and hence these finer particles will be carried further by currents than particles of a similar size not bound together by secretions

(Appendix III, Table 30; row 5, p. 546). The secretions of N. diversicolor affect all size ranges - both coarse and fine, and so resuspended particles of all sizes bound together by the secretions of this species will be carried further.

Variations in the effects which different species have on sedimentation might be explained by structural differences between the secretions. However, careful examination of the structural differences seen in the scanning electron microscope showed no very obvious causal relationship.

Scoloplos armiger secretions had no effect on sedimentation and I was unable to obtain scanning electron microscope pictures of them; this could be due to the technique I used which may have broken the structure of any secretions that the species produced. Schäfer (1972) states that S. armiger does not have a permanent dwelling tube, but while burrowing it constantly secretes mucus which does not consolidate the burrow wall. It therefore seems that S. armiger secretions may have a very delicate structure which was lost in my preparatory scanning electron microscope procedures, and which in the sedimentation experiments could have been destroyed during the inversion of the cylinder. They would therefore have had no effect on sedimentation.

(II) Effect of enzymes on animal secretions

Comparisons between the enzymes showed that their effects were different (Table 64, p. 369). Table 70 shows the particular substrate and the chemical bonds catalysed by each enzyme. The smaller the weight of secretions remaining after enzymic digestion, the more effective is the enzyme on that particular secretion. In general, since α -amylase was the most effective enzyme and catalyses the hydrolysis of 1 \rightarrow 4 glucosidic linkages of polysaccharides, this bond must be very common in the secretions. A converse argument applies

TABLE 70. Representative references on the different chemical bonds of substrates attacked by the different enzymes.

| Enzymes | Chemical bond attacked by the enzyme | Substrate | Reference | | |
|-------------------|---|--|---------------------|------|---------|
| | | | Author | Year | Page |
| α -amylase | α -1,4 glucosidic linkages of polysaccharides | Starch and glycogen | Lehninger | 1975 | 264-266 |
| | | | Stryer | 1975 | 376-377 |
| | | | Windholz | 1976 | 81 |
| Hyaluronidase | β (1 \rightarrow 4) linkages of hyaluronic acid | Hyaluronic acid | Lehninger | 1975 | 273 |
| Lipase | hydrolysis of the ester linkages of triglycerides | Fat | Fruton and Simmonds | 1953 | 512 |
| | | | Windholz | 1976 | 720 |
| Lysozyme | (β (1 \rightarrow 4) glycosidic bond) The glycosidic bond between C-1 of N-acetylmuramic acid (NAM) and C-4 of N-acetylglucosamine (NAG) of the cell-wall polysaccharides of bacteria | Cell-wall polysaccharide in bacteria | Stryer | 1975 | 136 |
| | | | Lehninger | 1975 | 270-271 |
| Pepsin | Peptide linkages in which an aromatic amino-acids provides the amino group for the sensitive peptide bond | Proteins | Fruton and Simmonds | 1953 | 128 |
| | | | Lehninger | 1975 | 106 |
| | | | Windholz | 1976 | 927 |
| Trypsin | Splits peptide bonds on the carboxyl side of lysine and arginine residues only (proteins to amino acids) | Proteins, also esters and amides containing the bond | Lehninger | 1975 | 106 |
| | | | Stryer | 1975 | 104 |
| | | | Windholz | 1976 | 1256 |

to the less effective enzymes.

There were also differences in the effects of the enzymes on the different species secretions. For example, with C. volutator, the most effective enzymes were α -amylase and trypsin and the least effective was lipase. Conversely with P. elegans secretions, the most effective enzyme was lipase and the least effective was hyaluronidase. This means that in the secretions of C. volutator, α 1 \rightarrow 4 glucosidic linkages of polysaccharids and the peptide bonds are the most common bonds, and the linkages of triglycerides are uncommon. By similar reasoning, triglyceride linkages were most common in the secretions of P. elegans and the β (1 \rightarrow 4) linkages of hyaluronic acid least common.

Comparisons between the secretions of the four species showed that N. diversicolor and C. volutator have the least resistance towards enzymic digestion and A. marina secretions were the most resistant (Table 65, p.370). This is significant ecologically. Bacteria may produce different types of enzymes in sediments, in which case A. marina secretions will be the most resistant to bacterial degradation and those of N. diversicolor and C. volutator the least resistant.

(III) How enzymic digestion alters the effect that secretions have on sedimentation

The results of this part can be summarised as follows. The maximum and minimum suspended dry weights of sediment were obtained from the top and bottom controls. The suspended dry weights obtained from sediment containing animal secretions after enzymic digestion were intermediate. The more effective the enzymes were, the closer were the suspended weights to the bottom control.

The results of this part supported the results from parts I and II. The more secretions on sediment grains, the slower these grains sediment, and the less secretions on sediment grains, the faster they sediment.

(IV) Refinement of the sedimentation procedure

The most important point demonstrated in these refined sedimentation experiments is that animal secretions cause greater weights and larger particles to be found towards the middle of the column.

The ecological consequences of these effects may be highly significant. There are at least three factors that may determine how far resuspended sediment particles are carried: the first is the binding ability of the secretions; the second is the current speed - faster currents will transport particles further; the third is the height above the estuarine bed to which the particles are initially suspended.

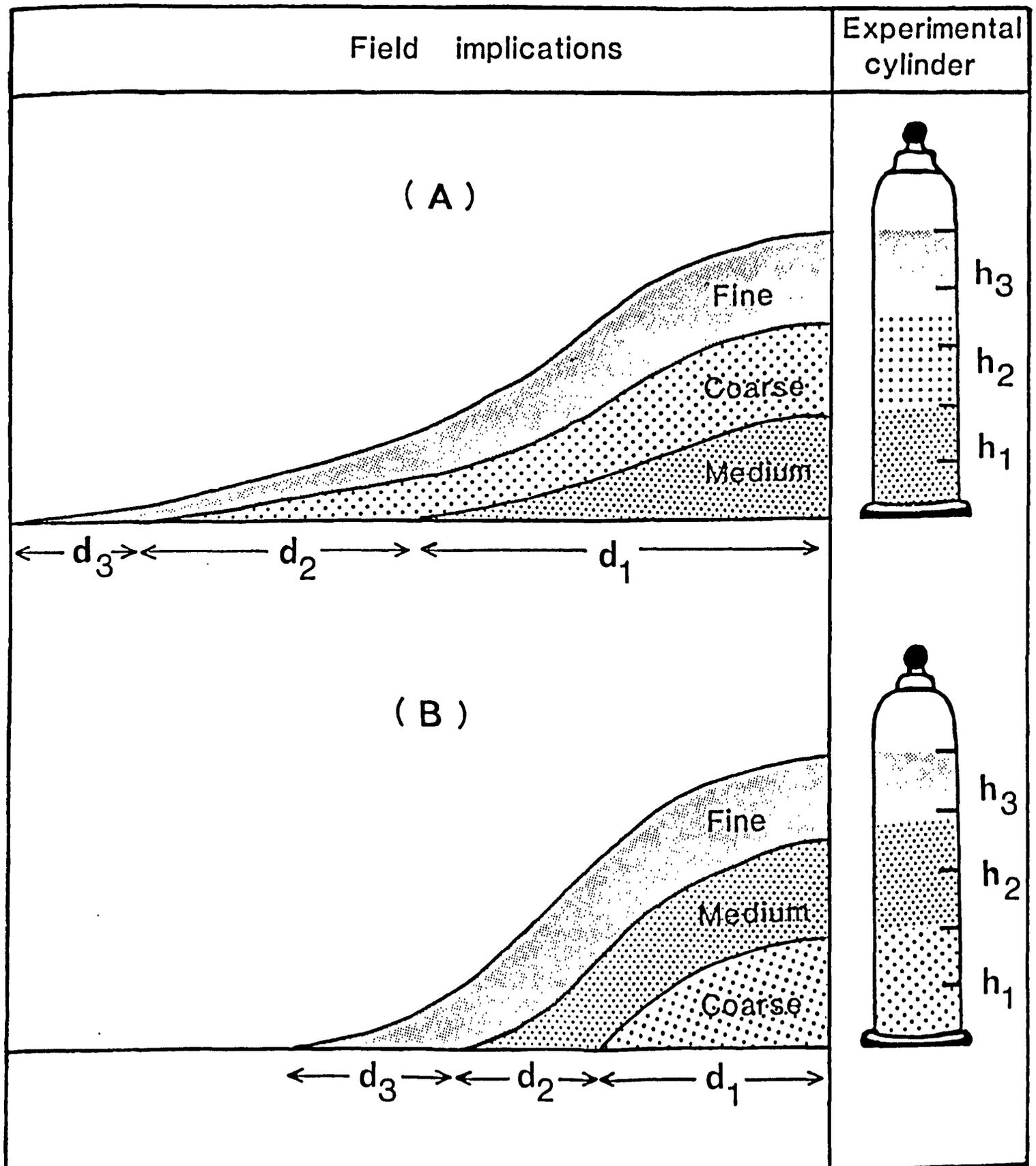
Only the first of these is directly relevant to this section. Consider two types of sediments having the same particle size distribution - one containing animals that produce burrows held together by secretions, and the other not (Figure 66). Water currents erode both sediments causing sediment suspension. These water currents will transport the suspended particles from their place of origin. The sediment particles bound together by animal secretions will settle more slowly and therefore will be transported further than the sediment particles containing no animal secretions (Figure 66).

In addition, there will be a particle size effect as illustrated in Figure 66. In the control sediment (Figure 66B), there is a gradual increase in particle size towards the bottom of the water column (Figure 66B, p. 427; Appendix III, Table 30, p. 546).

Therefore there will be a decrease in particle sizes with increasing distances (d_1-d_3) from where the particles were suspended initially. In the sediment treated with animal secretions coarse particle sizes are most abundant in the middle of the column (Figure 66A, h_2) (Figure 63, p. 412; Appendix III, Table 30, p. 546). This will cause the larger sized particles, which in the control cylinder settle at d_1 (Figure 66B), to be carried a further distance horizontally over the estuarine bed to d_2 (Figure 66A).

Figure 66

Diagram illustrating the horizontal distances (d_1 , d_2 , d_3) to which particles suspended at different heights (h_1 , h_2 , h_3) would be carried by horizontal water currents in an estuary. A: secretions present; B: secretions absent. ( ,  , ) = particle sizes.



Comparisons between conclusions of Part I and Part IV

I would like to comment on two statements I made in part I and part IV of the present discussion (pp. 419 and 424). These statements refer to the effects that animal secretions have in reducing sedimentation of different sized particles.

In part I, secretions of A. marina followed by those of N. diversicolor had most effect on sedimentation at the 5- and 10-second time intervals, and secretions of N. diversicolor followed by those of C. volutator had most effect at 40, 60 and 120 seconds (Table 59, p. 340). This means that on comparing the three species, A. marina has the most effect in reducing sedimentation of large particles, C. volutator affected the small particles more, and N. diversicolor affected both large and small particles. However, all three species markedly reduced the sedimentation at all time intervals when compared with the controls, and hence caused particles of all sizes to sediment more slowly than in the controls.

In part IV, the secretions of C. volutator and N. diversicolor caused greater weights of sediment containing larger sized particles to be found in the middle of the column than in the controls (middle of the column = 10 cm from the water surface vertically). This depth (10 cm) is exactly the same depth at which the samples were taken in part I, and the increased weights found in part IV when compared to the controls are the same as the increased weights found in part I for the same two species at the same depth, even though the experiments were conducted twenty-seven months apart (Table 71).

TABLE 71. Sedimentation experiments: weights of sediment with animal secretions in suspension (mg dry weight/ml) at different time intervals, at 10 cm depth. n = number of replicate samples. 19.12.1981 and 5.3.1984 are the dates at which the two series of experiments were conducted. The figures represent the means and standard deviations of the replicate samples. Data from Appendix III, Table 2, p. 518; rows 1, 2 and 3, and Appendix III, Table 19, p. 535; rows 2, 5 and 8.

| Time (secs) | Secretions of <i>C. volutator</i> | | Secretions of <i>N. diversicolor</i> | | Control | |
|----------------|--------------------------------------|-----------------------|---|-----------------------|-------------------------|-----------------------|
| | 19.12.81 ($n = 5$) | 5.3.84 ($n = 2$) | 19.12.81 ($n = 5$) | 5.3.84 ($n = 2$) | 19.12.81 ($n = 5$) | 5.3.84 ($n = 2$) |
| 5 | 62.83 + 0.06380 | 62.28 + 0.6010 | 92.78 + 0.05595 | 90.76 + 2.015 | 49.83 + 0.05595 | 49.92 + 0.09192 |
| 10 | 50.35 + 0.07071 | 50.18 + 0.2828 | 80.03 + 0.01789 | 77.54 + 3.550 | 31.78 + 0.07950 | 31.86 + 0.1061 |
| 20 | 48.93 + 0.05595 | 46.63 + 3.182 | 64.78 + 0.03962 | 62.46 + 0.9546 | 5.275 + 0.07289 | 5.263 + 0.1237 |
| 40 | 36.59 + 0.1545 | 36.33 + 0.3182 | 50.15 + 0.05595 | 50.11 + 0.0354 | 4.925 + 0.03953 | 3.300 + 0.07071 |
| 60 | 21.65 + 0.06380 | 21.57 + 0.02121 | 38.88 + 0.01789 | 38.68 + 0.07070 | 2.150 + 0.03953 | 2.113 + 0.01768 |
| 120 | 7.825 + 0.08839 | 7.525 + 0.03536 | 15.03 + 0.03962 | 12.54 + 3.521 | 1.020 + 0.03708 | 0.8750 + 0.1768 |

SECTION III

Summary(I) Effect of animal secretions on sedimentation

1. C. volutator and N. diversicolor from Langbank, and P. elegans, S. armiger and A. marina from Ardmore were selected to test the effects of their secretions on sedimentation.
2. The species were tested against their own sediment and against Rockware sediment.
3. Animals were maintained in sediment for seven days before conducting sedimentation experiments.
4. Control dishes (with no animals) were also maintained.
5. Sedimentation experiments were conducted using the definitive method (Section II, p. 200). A known weight of sediment was mixed in a known volume of synthetic sea water by inverting the cylinder three times. Five replicate samples were then removed at successive time intervals (5, 10, 20, 40, 60 and 120 seconds).
6. In general, the secretions decreased sedimentation compared with the control sediment; S. armiger secretions were the only exception.
7. With Langbank species, the secretions of N. diversicolor had a greater effect than those of C. volutator. The effect of the mixed species (i.e. C. volutator and N. diversicolor) was greater than that of the single species.
8. With Ardmore species, the secretions of A. marina had a greater effect than those of P. elegans. S. armiger secretions had no effect.

9. Secretions decreased sedimentation of the species' natural sediments (i.e. Langbank or Ardmore sediment) more than Rockware sediments.
10. The mean suspended weight obtained during sedimentation of the four effective species were ranked to show which species produced the greatest effect. Interesting changes in ranking occurred with time. At the beginning of the experiment (5, 10 seconds), A. marina secretions followed by N. diversicolor had the greatest effect. However, towards the end of the experiment (40, 60 and 120 seconds), N. diversicolor followed by C. volutator had the greatest effect. This may be associated with particle size.
11. Scanning electron microscope photomicrographs showed that secretions produced by the species have different structures.
12. The secretions of N. diversicolor are thinner than those of C. volutator and leaf-like structures can be clearly seen. Small beads are obvious along the length of the threads of both species.
13. Secretions of P. elegans form a tight criss-cross lattice which is net-like. The secretions of A. marina consist of denser sheets of bands or cords.

(II) Effect of enzymes on animal secretions

1. The digestive effects of six enzymes - α -amylase, hyaluronidase, lipase, lysozyme, pepsin and trypsin were tested on secretions of C. volutator, N. diversicolor, P. elegans and A. marina.
2. The enzymes had different effects on the four species' secretions.
3. With C. volutator secretions, the most effective enzymes were α -amylase and trypsin, followed by hyaluronidase, pepsin, lysozyme and lipase, in that order.

4. With N. diversicolor secretions, the most effective enzyme was α -amylase, followed by pepsin, lysozyme, lipase, trypsin and hyaluronidase.
5. With P. elegans secretions, the most effective enzyme was lipase followed by α -amylase, lysozyme, pepsin, trypsin and hyaluronidase.
6. With A. marina secretions, α -amylase was the most effective enzyme followed by pepsin, lipase, lysozyme, hyaluronidase and trypsin.
7. Comparison between the enzymes showed that in general the most effective enzyme was α -amylase followed by pepsin, lipase, lysozyme, trypsin and lastly hyaluronidase.
8. Comparison between the different types of secretions showed that N. diversicolor secretions had the least resistance towards enzymic digestion, followed by C. volutator and P. elegans secretions. A. marina secretions were the most resistant towards enzymic action.

(III) How enzymic digestion of secretions alters the effect that the secretions have on sedimentation

1. Secretions from the four burrowing benthic species (i.e. C. volutator, N. diversicolor, P. elegans and A. marina) were tested separately with the six enzymes (i.e. α -amylase, hyaluronidase, lipase, lysozyme, pepsin and trypsin). The weights of secretions remaining after enzymic digestion were then used in conducting sedimentation experiments.
2. Two types of controls were used with each species' secretions - a top control (sediment with secretions) and a bottom control (sediment without secretions). Eight sedimentation cylinders were set up for each species' secretion: six for the six enzymes, and two for the two controls.

3. The maximum and minimum values of suspended weights were obtained from the top and bottom controls respectively. The suspended weights obtained after enzymic digestion were between the top and bottom controls.
4. The effects of the enzymes on sedimentation were similar to their effects in digesting secretions (Part II, p.355) when compared by ranking. Because of this similarity, the results of this part were compared statistically with those of Part II.

Linear regressions were conducted on the weights of secretions remaining after enzymic treatments (x) - Part II, against the suspended weights obtained from the sedimentation experiments (y) - Part III. This gave twenty-four regressions (four species x 6 times = 24), eighteen of which were significant, with positive correlations (i.e. positive slopes) between the two variables.

(IV) Refinement on sedimentation procedure

1. The 5-second sedimentation readings in the secretion experiments (mg dry sediment/ml) were significantly higher than that expected from an even distribution of the sediment at the beginning of the sedimentation experiment. Experiments were conducted to find out why this was so.
2. The experiments were carried out with sediment treated with C. volutator secretions and N. diversicolor secretions, and with control sediment with no secretions. Rockware sediment was used throughout.
3. Two millilitre samples were taken at 5, 10, 20, 40, 60 and 120 seconds at three different depths - 5, 10 and 20 cm.

4. Weights and particle sizes (Feret diameter) were determined on the suspended sediment thus obtained. The particle size analysis involved measurements of 5400 individual sand grains.
5. For sediment treated with N. diversicolor secretions and C. volutator secretions, the weights and particle sizes were greatest at 10 cm, followed by those at 20 cm and then those at 5 cm.
6. For the control sediment, the weights and particle sizes were greatest at 20 cm, followed by those at 10 cm and then those at 5 cm.
7. The results were analysed statistically by analyses of variance and student t tests, and all the statements in 5 and 6 were statistically substantiated.

