INORGANIC PARTICULATE SUSPENSIONS AND THE FEEDING OF ASCIDIANS

(one volume)

Ian John Robbins

A thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy, following research conducted at the University Marine Biological Station, Millport, Scotland.

November, 1981

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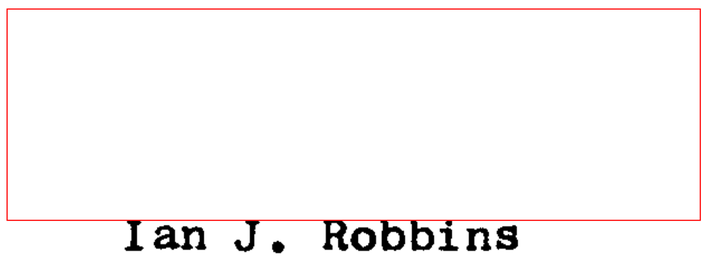
Cette thèse est dédiée à ma femme Françoise

DECLARATION

I hereby certify that the work embodied in this thesis, for the degree of Doctor of Philosophy, is a result of my own work, which has not previously been submitted for any degree.



Ian J. Robbins

I certify that this study has been performed under my supervision.

Dr. P.G. Moore

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My thanks are especially due to my supervisor, Dr. P.G. Moore, for his encouragement and advice throughout the project.

I would like to thank the following colleagues at Millport for helpful advice and criticism; Professor J. Allen, Dr. R.J.A. Atkinson, Dr. C. Richardson, Dr. J.G. McHenery, Dr. V. Smith, Dr. M. Benwell, Dr. P. Schembri, Dr. R. Nash and Miss H. Grigg.

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SUMMARY

1) The effects of inorganic particulate suspensions on the feeding processes of the ascidians Ciona intestinalis (L), Ascidiella scabra (Müller) and Ascidia mentula (Müller) were investigated. The morphology of A. scabra was dependent upon the locality of its origin (mud substrata or the fronds of Fucus serratus L.). The two morphs were treated separately and are referred to as A. scabra (mud) and A. scabra (Fucus).

2) The filtration rates of C. intestinalis and A. scabra are inversely related to inorganic particulate suspension concentration. The decline is such that the ingestion rate reaches a maximum and thereafter remains constant.

3) Filtration rates are higher for larger individuals of C. intestinalis. Increasing concentrations of inorganic particulate suspensions cause a similar decrease in filtration rate for all size groups. The maximum ingestion rate increases with body size.

4) The rate of spontaneous squirting increases with increased seston load (regardless of whether the seston is organic or inorganic). The increase is initiated once the gut becomes satiated and is suggested to be a mechanism by which overloading of the gut is avoided.

5) Under most conditions, the duration of squirts remained constant, such that the time lost on account of squirting is a function of particulate suspension concentration, once the gut is satiated.

6) The time loss due to squirting is not sufficient to fully explain the reductions in filtration rate observed. A reduction in the absolute pumping rate of A. mentula was discovered and suggested as a solution to the above discrepancy.

7) Feeding in C. intestinalis is a continual process, the passage of the mucus food cord being little affected by suspension concentration. The faeces have a similar form regardless of the quantity or quality of material ingested. The faeces are composed of sinuous folds of the food cord sandwiched between peritrophic membranes. The proximity of these folds is dependent upon ingestion rate.

8) Assimilation efficiency (for any particular food type) is a function of total ingestion rate. Inorganic particulate suspensions have the effect of increasing this rate (and hence reducing the assimilation efficiency) or, if the gut is satiated, reducing the quantity of organic material ingested by a process of 'dilution'.

9) The sum effect of inorganic particulate suspensions is detrimental to the processes of feeding in ascidians. Growth is retarded and, in high concentrations, mortality induced.

GENERAL INTRODUCTION

i. Inorganic suspended matter and the feeding of ascidians

Ascidians, being filter-feeding animals, are affected by the amount and nature of suspended matter in the surrounding water, but there are few observations to indicate the importance of this factor in nature (Millar, 1971a). Millar (1971b) has also suggested that the usual site of fouling by ascidians (i.e. near the surface of the sea) is favourable to growth by providing water of relatively high temperature during the growing season, and with comparatively few suspended inorganic particles which interfere with efficient feeding.

Circumstantial evidence of the effects of turbidity on ascidians can be gained from their distribution (reviewed by Moore, 1977). It would appear that many species are excluded by turbid waters and that sheltered sites with clear water are marked with luxurious growth of ascidians. One must regard these data with caution, however, as other environmental factors are likely to vary between sites investigated. C. Monniot (1965, cited by Millar, 1971a) observed that excessive concentrations of suspended material led to the clogging of the branchial mechanism of Pyurid ascidians, and caused death. Monniot further recorded the destruction of an abundant population of Ascidia sp. following disturbance of the sea-bed and increased turbidity.

Since most ascidians feed unselectively of quality, the potential impact of inorganic particles in quantity may be presumed to be generally detrimental to feeding (Moore, 1977). There are, however, no laboratory studies specifically aimed at elucidating the effects of inorganic particulate suspensions on the feeding of ascidians.

ii. The feeding process in ascidians

The method of filter-feeding of ascidians is well documented (MacGinitie, 1939; Jørgensen, 1954, 1966; Werner and Werner, 1954; Fenaux, 1968) and a full description is unwarranted here. A brief description, however, will serve to put the following work into context.

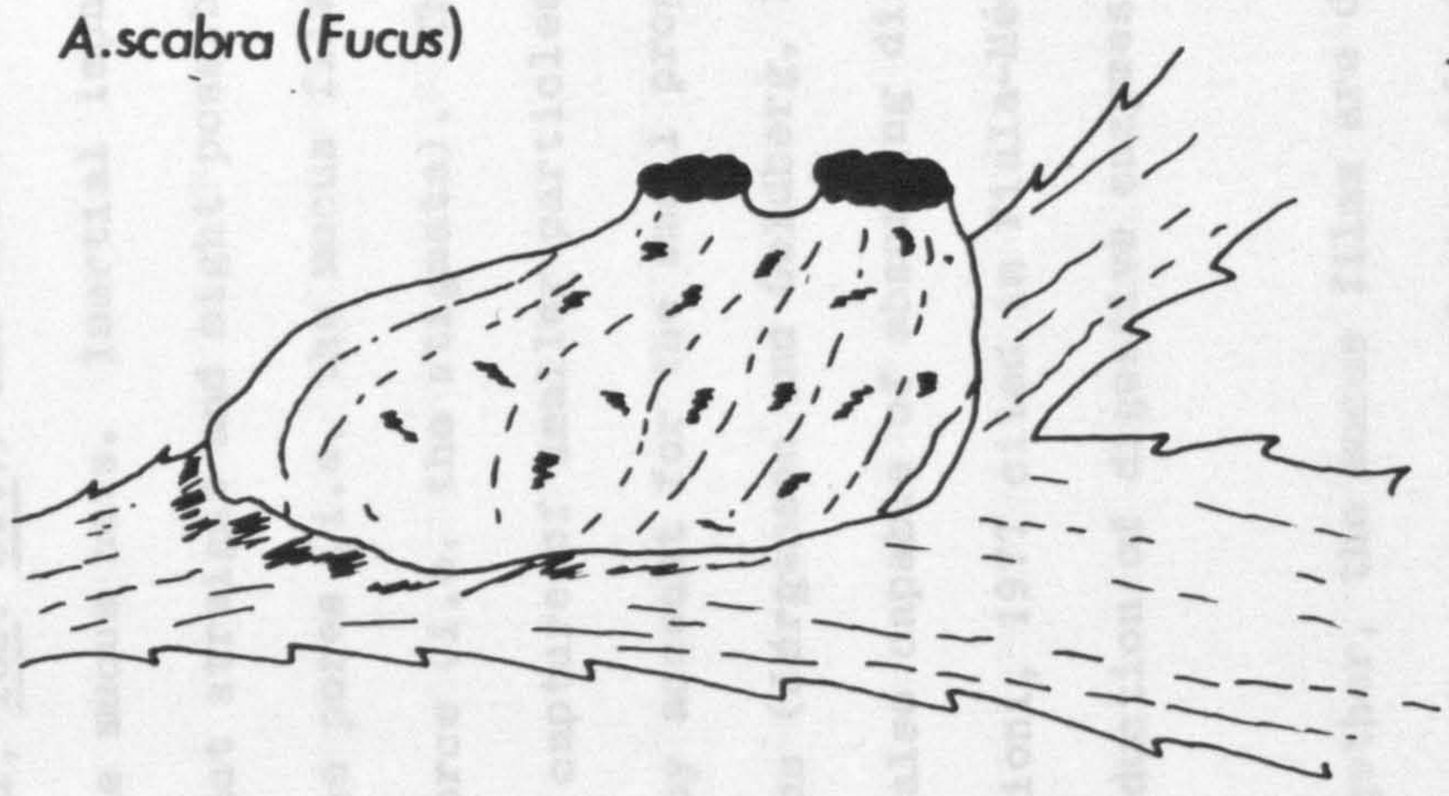
Water is pumped through the pharynx by the cilia lining the inside of the stigmata beating in dexioplectic metachronal waves (Takahashi, et al., 1973). Mucus films, continuously produced by the endostyle, are passed across both of the inner faces of the pharynx. They are, thence, rolled together by the languets of the dorsal lamina and drawn into the oesophagus by ciliary action.

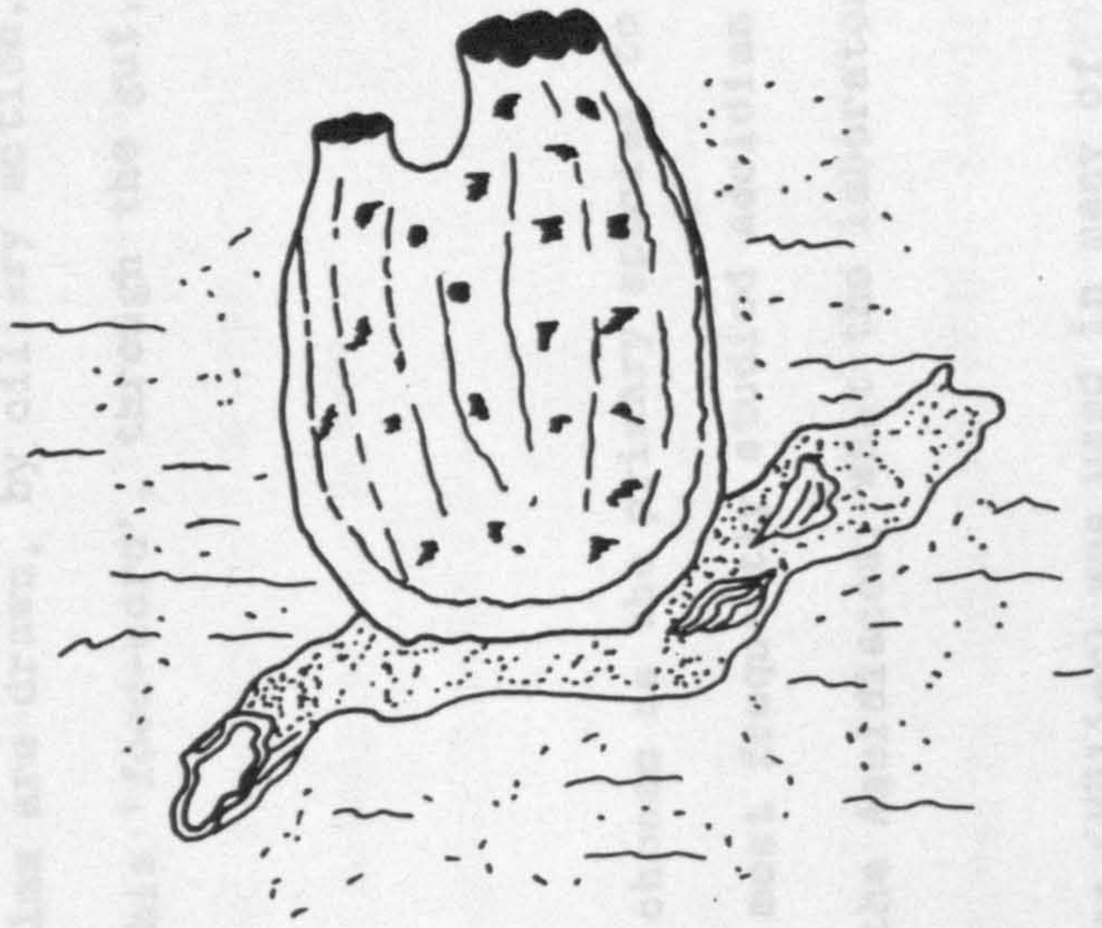
The mucus films serve to filter particles from the water passing through the pharynx. The films consist of filaments with fine rectangular meshes in between (Flood and Fiala-Médioni, 1979; F. Monniot, 1979). The pore sizes of this net are reportedly 0,42 x 0,75µm in Ciona intestinalis (Flood and Fiala-Médioni, 1979). But as these authors point out, fixation of specimens for electron microscopy causes some shrinkage and, in nature, particles are only retained with 100% efficiency when larger than 1-2µm (Randløv and Riisgård, 1979).

Rubenstein and Koehl (1977) have detailed five mechanisms by which pore filters may capture particles other than by sieving. These are a) direct interception, b) inertial impaction, c) gravitational deposition, d) diffusion or motile-particle deposition, e) electrostatic attraction. Due to the small size of the pores in the mucus mesh, sieving and direct interception must be the major mechanisms involved. If sieving were the sole mechanism, the retention efficiency would be expected to drop to zero for particles smaller than the pore size of the mesh. This is not the case (Randløv and Riisgård, loc. cit.) and some of the other

FIGURE 1 The morphology of A. scabra from two habitats.

A.scabra (Fucus)





A.scabra (Mud)

mechanisms must come into play. Gravitational deposition and diffusion or motile-particle deposition are associated with long, narrow pores (Rubenstein and Koehl, loc. cit.) and are unlikely to be of much consequence with fine mucus nets. Inertial impaction can be initiated when the pores are not straight and might possibly be induced in a similar manner by the pores (i.e. the mucus film) moving relative to the water driving force (i.e. the stigmata). This method may therefore, be important in the capture of smaller particles by ascidians. Electrostatic attraction may account for the small proportion of proteins retained by ascidians (Jørgensen and Goldberg, 1953).

The pharynx is also capable of absorbing dissolved organic matter directly (Fiala-Médioni, 1977 cited in Fiala-Médioni, 1978d) and it is involved in the production of digestive enzymes (Fiala-Médioni and Pequignat, 1975).

Once rolled together, the mucus films are drawn, by ciliary action, into the oesophagus. The passage of this 'food-cord', through the gut, is described in detail in Chapter 5.

iii. The ascidians chosen for study

Ciona intestinalis (Linnaeus) was chosen as the primary species to be investigated. It is, perhaps, the most frequently studied ascidian and might be considered as being, to the Ascidiacea, what the laboratory rat is to the Mammalia.

A second species, Ascidiella scabra (Müller) was used in many of the experiments. This species was chosen because it can be found, not only intertidally on fronds of Fucus serratus (L), but also subtidally, attached to shells and debris scattered on mud substrata. Animals from the latter environment might be expected to experience greater

concentrations of suspended inorganic particulates. Taylor-Lindsay and Thompson (1930) reported that there was no uniformity in the degree of attachment of A. scabra, the attachment ranging from 'basal' to 'whole side'. Taylor-Lindsay and Thompson dismissed the earlier claim of Herdman (1893) that the two extremes can be separated specifically, A. virginea being more erect and A. scabra recumbent. They regarded the recumbent form as occurring where the substratum afforded the opportunity and where a large average size was not attained.

Measurements of the angle of attachment (∅) show that the animals from the mud substratum tend to be more erect than those from F. serratus fronds (Fig. 2). The two morphs might also exhibit different reactions to the presence of inorganic particulate suspensions. They are hereafter referred to as A. scabra (Mud) and A. scabra (Fucus) and are illustrated in Fig. 1.

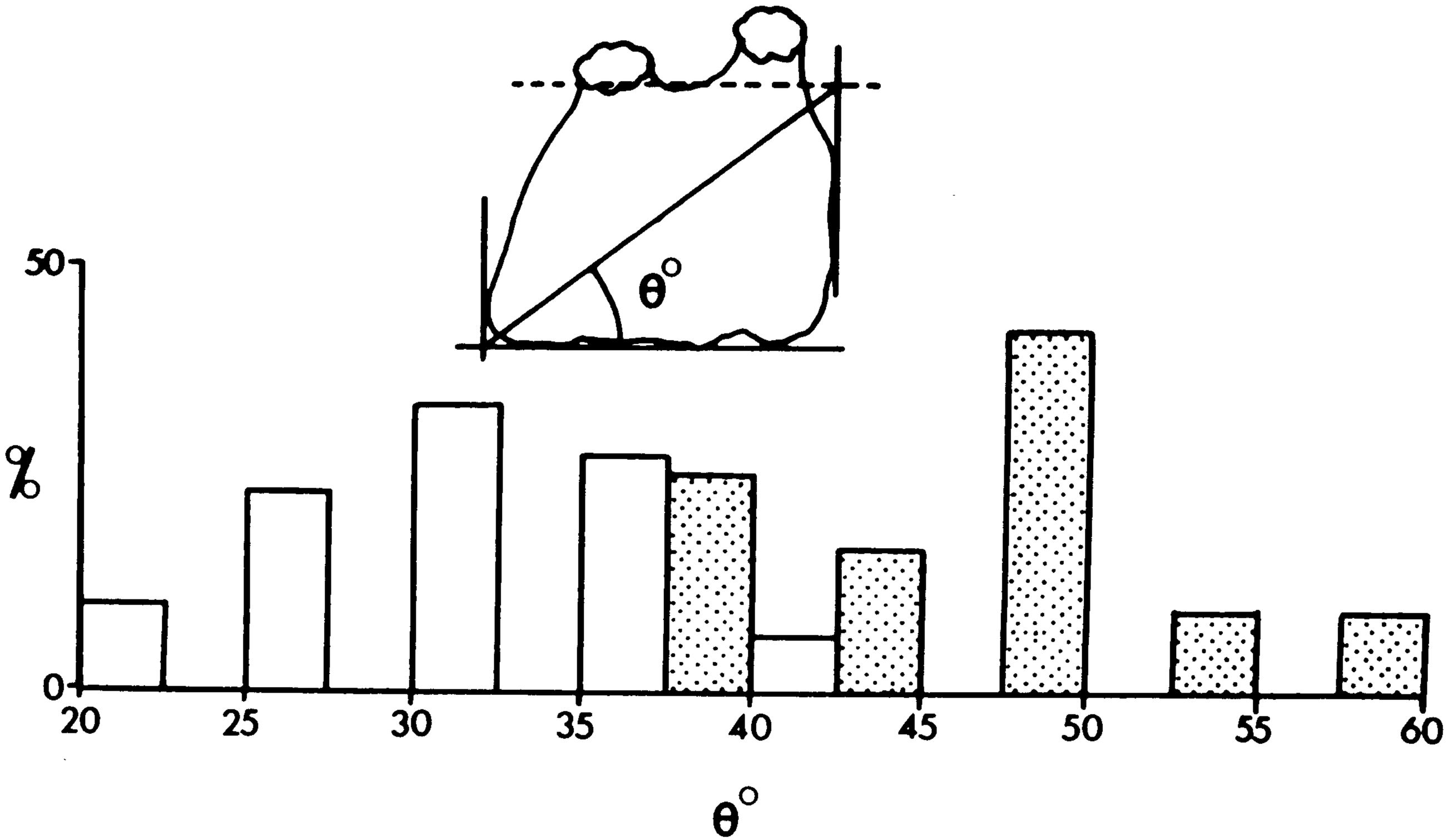
Ascidia mentula (Müller) was chosen for use with the split-chamber constant-level apparatus (Chapter 4). It was found suitable on account of its large size and the smoothness and hardness of its test.

All three ascidian species studied belong to the sub-order Phleobranchiata and have similar pharyngeal morphology.

iv. Inorganic particulate suspensions in the marine environment

The background level of turbidity in seawater varies greatly (Moore, 1977). Recorded levels have been reviewed by Jørgensen (1966), Jacobs and Ewing (1969), Chester and Stoner (1972) and Moore (1977). Average suspended loads for inshore waters are 1,0 $mg.l^{-1}$ (Chester and Stoner, 1972; Moore, 1977), but values of several hundred or several thousands of $mg.l^{-1}$ may occur along eroding shores and in certain estuarine situations. In addition, man's impingement on the oceans may increase

FIGURE 2 The angle of settlement (θ°) of A. scabra from mud (shaded) and Fucus (unshaded).


θ°
50
%
0
20
25
30
35
40
45
50
55
60
θ°

the suspended inorganic particulate levels several fold. Such activities as might cause these increases are a) effluent discharge (Green, 1972 cited in Moore, 1977); b) dredging (Mackin, 1962); c) marine mining (Padan, 1971) and d) marine construction operations.

v. Physical parameters of the inorganic particulates chosen for study

a. Particulates used in the study

Two commercially-available inorganic particulates were used in these studies, viz. Fuller's earth and Kaolin. They were chosen on account of their naturality and use by several other investigators.

In addition, the clay and silt fraction was separated from littoral mud from Ballochmartin Bay (Isle of Cumbrae, Scotland) - hereafter referred to as Ballochmartin mud. The mud was suspended in distilled water, filtered through a 125µm mesh screen and oven dried. The oven dried flakes of mud were ground down in a pestle and mortar.

b. Particle size distribution

Particle size distribution was analysed by the pipette method described by Folk (1974). Since this method can only be used for particles of less than 62µm in diameter, the inorganic particulates were, firstly, dry sieved to remove larger particles. It is unlikely that particles of diameter greater than 62µm would remain suspended for any appreciable length of time in the following experiments. Particles of less than 0,98µm diameter were not analysed, as ascidians are unable to retain them efficiently (Randlóv and Riisgård, 1979).

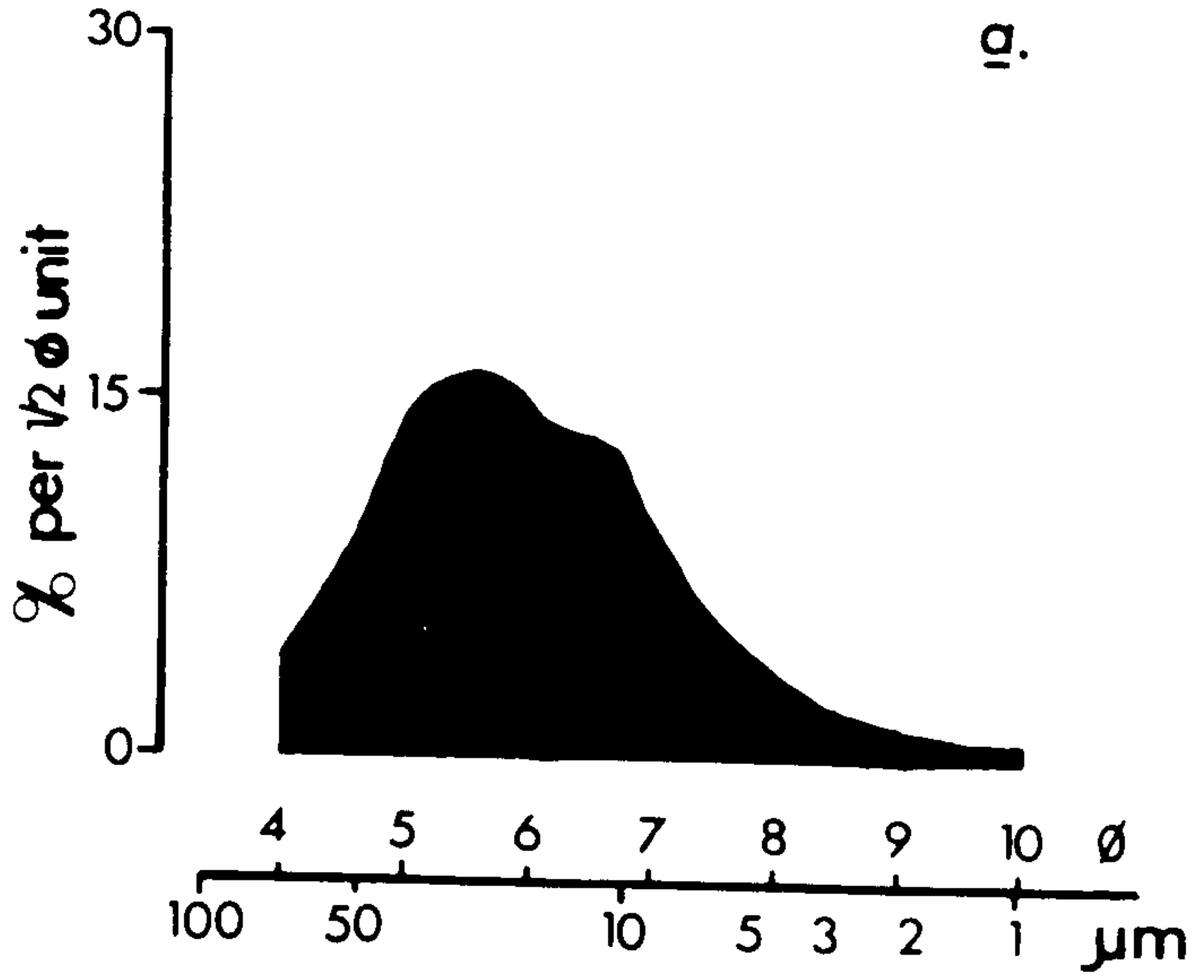
15gm of each particulate was suspended in 1l of distilled water in a measuring cylinder (kept at 28°C in a waterbath). 20ml samples were taken by pipette at depths and times calculated from the formula and Table given in Folk (1974). The samples were drained into preweighed

FIGURE 3 Physical parameters of Fuller's earth

a) Grain size distribution

b) Sphericity-form triangle. Data in percent.

a.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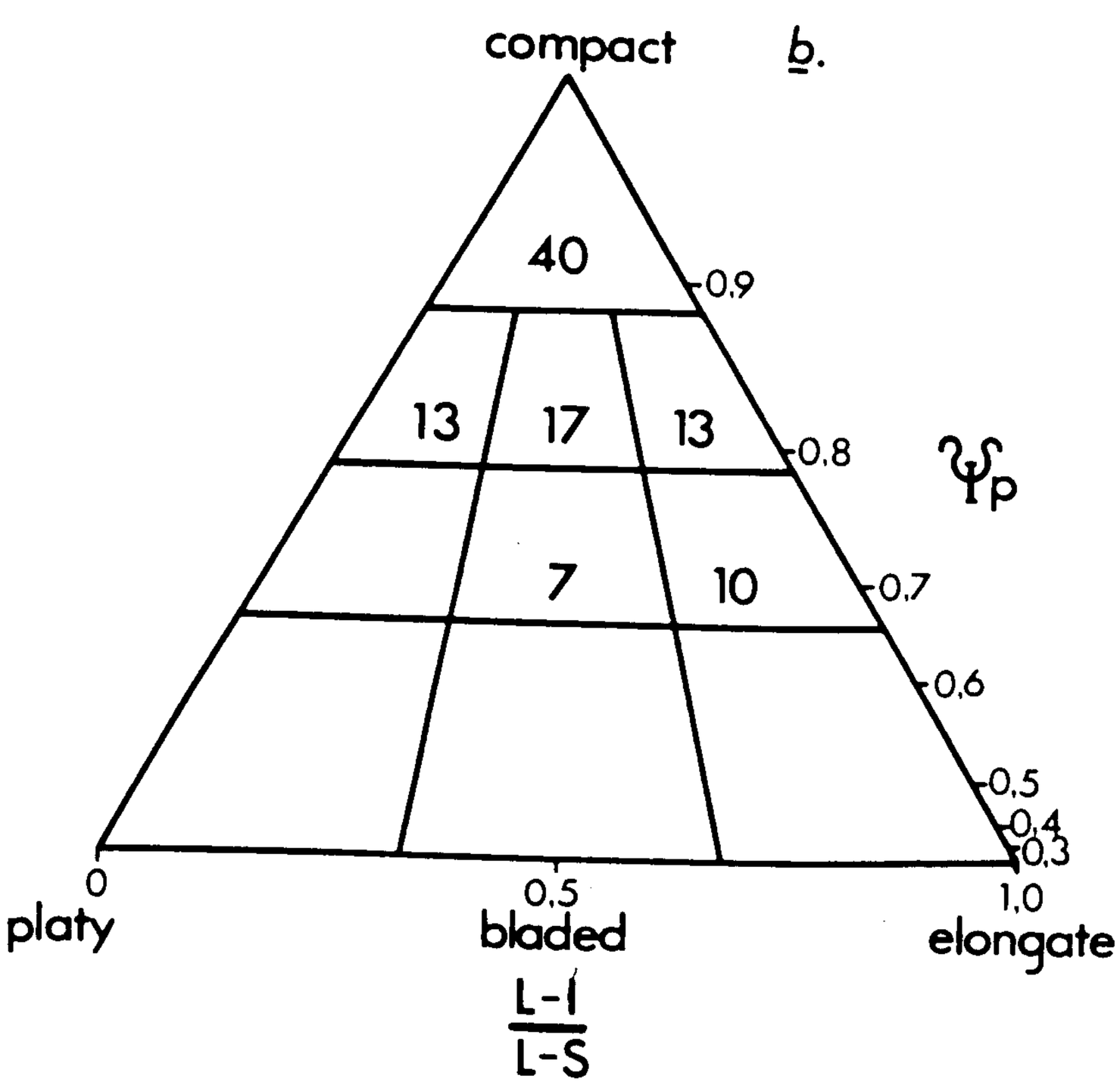
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platy
0.5
bladed
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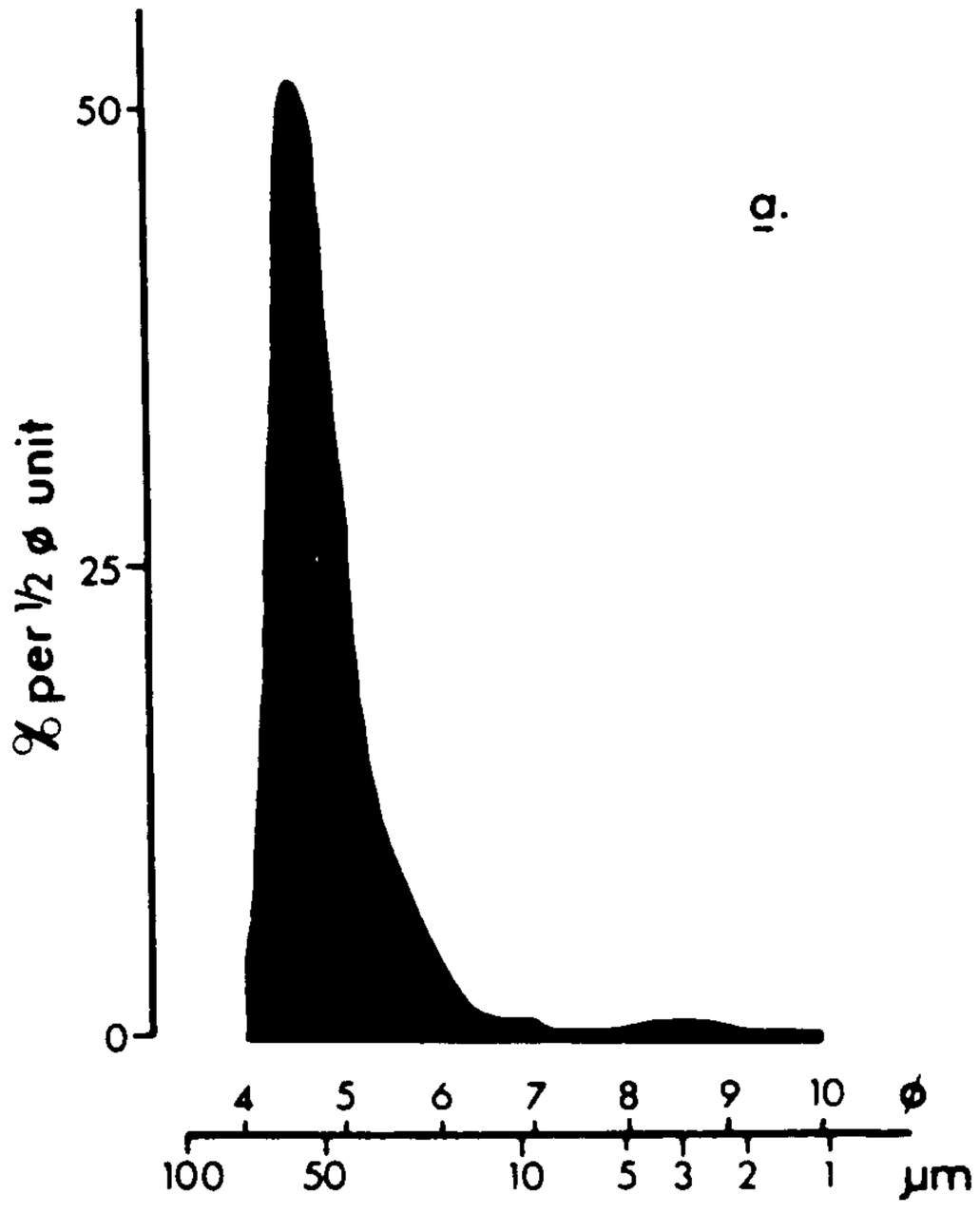
elongate
L-I
L-S

FIGURE 4 Physical parameters of Kaolin

a) Grain size distribution

b) Sphericity-form triangle. Data in percent.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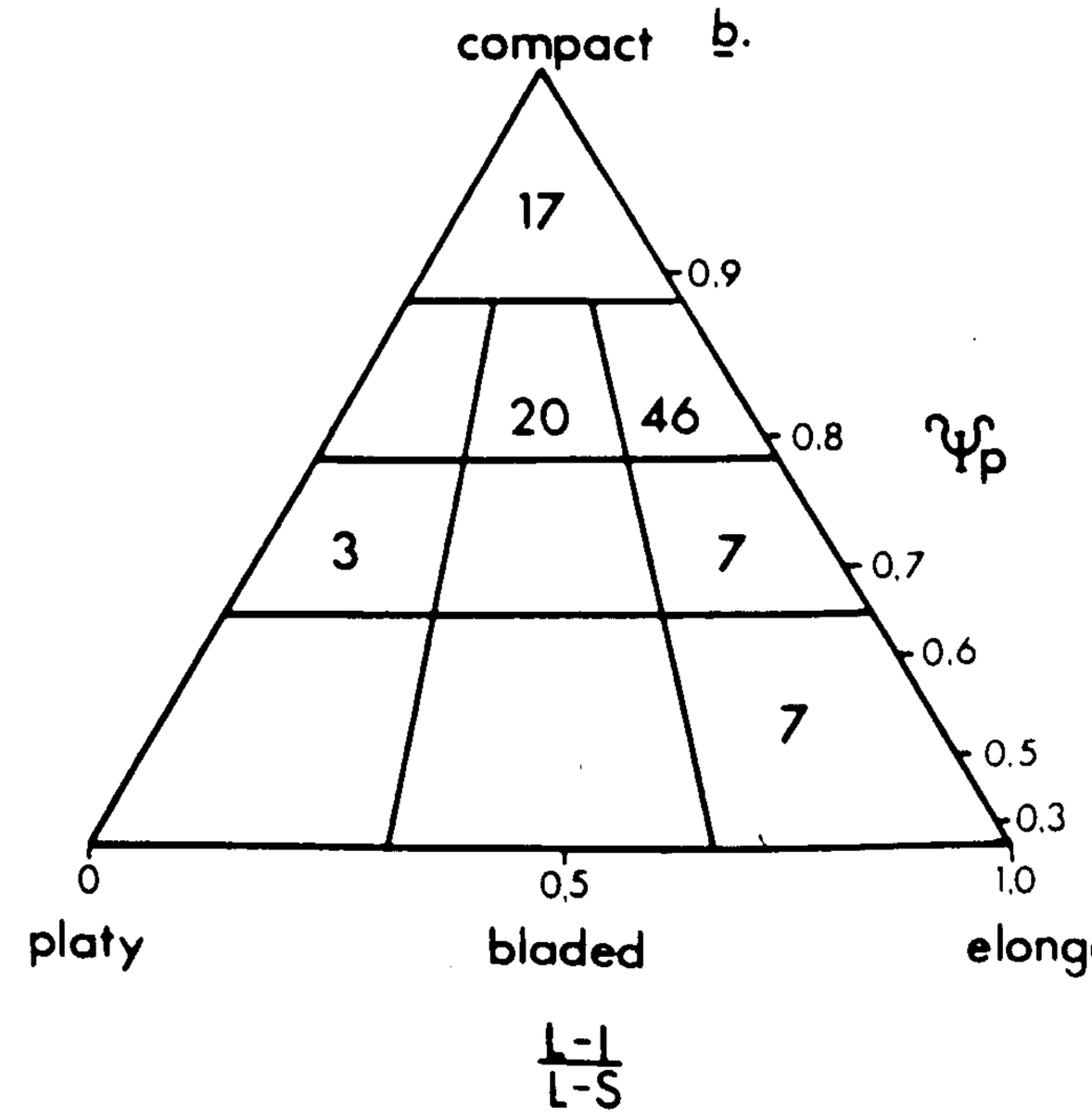
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0.5
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platy
bladed
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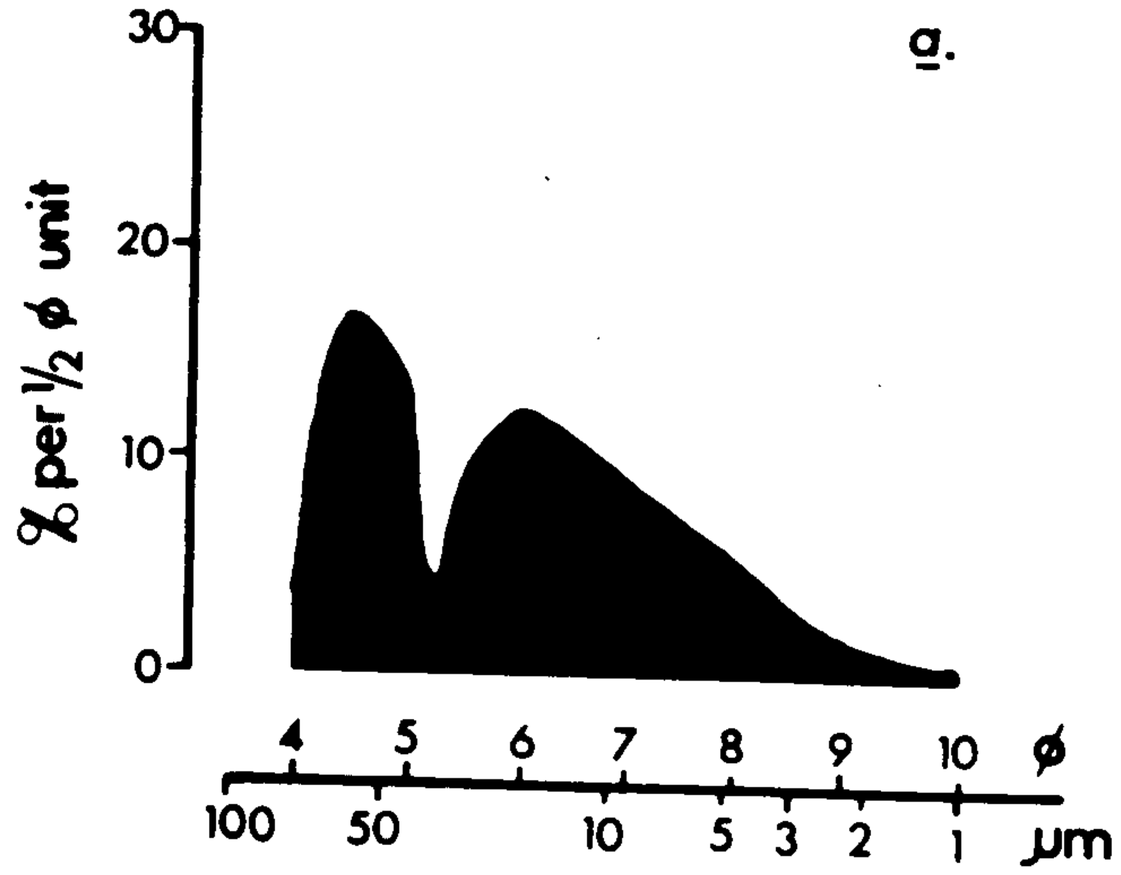
$\frac{L-I}{L-S}$

FIGURE 5 Physical parameters of Ballochmartin mud

a) Grain size distribution

b) Sphericity-form triangle. Data in percent.

a.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% per ½ ϕ Unit
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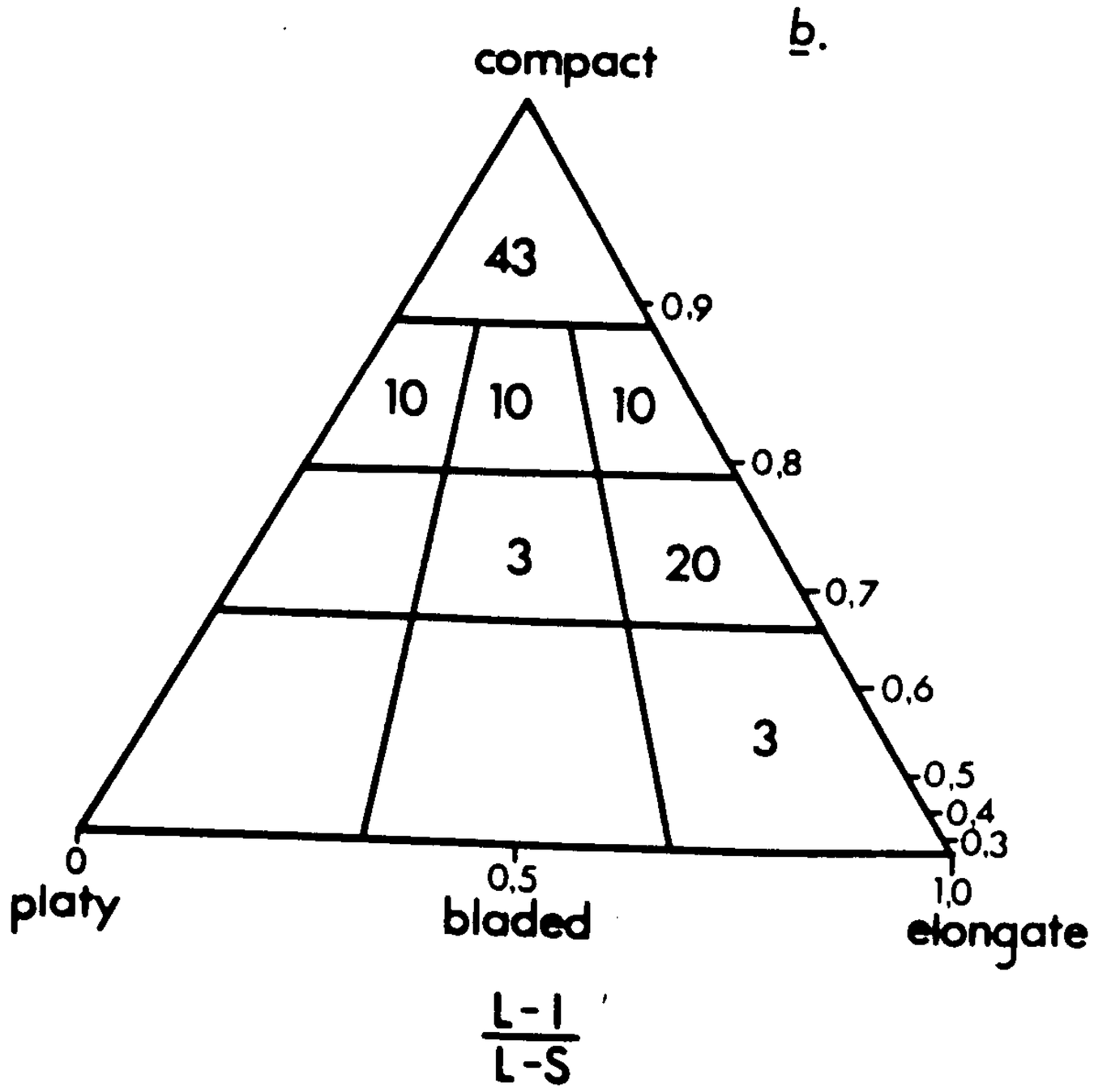
100 50 10 5 3 2 1 µm

b.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L-I
L-S

50ml beakers along with additional 20ml aliquots of distilled water used to wash out the pipette after each sampling. The beakers were covered with watch glasses and oven dried for 72 hours. They were allowed to equilibrate to the temperature and humidity of the balance room for 8 hours and then re-weighed.

Cumulative weight curves were constructed and data points taken from these to construct frequency curves.

c. Particle shape distribution

A small volume of each inorganic particulate suspension was placed in a cavity slide constructed from pieces of coverslip stuck onto a microscope slide with DPX mountant, to the specifications of Fairs (1951). The particles were examined under 400X magnification. Two measurements (viz. length and width) were made in the horizontal plane using a calibrated graticule set into the eye-piece of the microscope. A third measurement (viz. depth) was made in the vertical plane by focussing on the slide and on the top of the particle and noting the difference in readings on the focussing drum scale of the microscope. The measurements were arranged as long, intermediate and short (L, I and S). The effective setting sphericity (Ψ) was calculated from the equation,

$$\Psi = \sqrt[3]{\frac{S^2}{LI}}$$

This was plotted against the parameter,

$$\frac{L-I}{L-S}$$

on a sphericity-form triangle as described by Folk (1974). The percentages of particles falling within each portion of the triangle are given in Figs. 3-5b.

d. Particle numbers

Particle counts were made with a haemocytometer (Neubauer B.S. 748) using samples from a suspension of each particulate in filtered seawater. 100ml samples were taken simultaneously from these suspensions and filtered on Whatman GFC filter papers to ascertain the suspension weight. The particle counts are given as the number of particles.mg^{-1} of material in suspension (Table 1).

e. Particle packing volumes

Samples of suspensions, of known weight per litre, were drawn into microcapillary tubes that had previously been silicon coated (siliclad) to minimise particle adherence. The bottoms of the tubes were blocked off with ester wax, and the suspensions were allowed to settle in the tubes for 48 hours. The lengths of the tubes and the sediment pellets were measured and used to calculate the packing volume per mg of suspended material (Table 2).

f. Results

Fuller's earth and Ballochmartin mud both have a large spread of particle sizes. Fuller's earth has a modal particle size of 25-30μm. Ballochmartin mud has a bimodal distribution, the modes being at 15-20μm and 40-50μm. Kaolin shows a much more pronounced peak, representing a modal diameter of 25-35μm. The three particulate types have size spectra showing some similarity to naturally occurring inorganic particulates in certain coastal regions (Eisma and Kalf, 1979).

Fuller's earth has a mode of particles of a compact form with only a slight tendency towards being elongate. Kaolin has a mode of particles being slightly elongate. The bimodal size peak of Ballochmartin mud is reflected in the shape distribution, the larger size mode having a more elongate form than the compact smaller size mode.

Fuller's earth	$6,1.10^{6}$
Kaolin	$1,8.10^{8}$
Ballochmartin mud	$3,3.10^{7}$

TABLE 1 Particle counts of inorganic particulate suspensions (particles.mg^{-1}).

	Mean volume	Standard deviation
Fuller's earth	$3,17.10^{-3}$	$0,25.10^{-3}$
Kaolin	$5,07.10^{-2}$	$0,22.10^{-2}$
Ballochmartin mud	$2,33.10^{-3}$	$0,37.10^{-3}$

TABLE 2 Particle packing volumes ($cm^{3}.mg^{-1}$) of inorganic particulate suspensions.

The particle count of Kaolin is ten times higher than those of Ballochmartin mud and Fuller's earth. This is due to the extremely light nature of the particles. This lightness increases the packing volume, as the gravitational packing force is reduced.

vi. The algal cultures used in the study

The main algal species used was the unicellular flagellate Tetraselmis seucica (Kylin) Butch. In some of the earlier experiments, the very similar unicellular flagellate, Dunaliella salina (Dunal) Teodoresca was used.

The algae were grown in continuous monoculture by the British Steel Sedimentary Pollution Unit based at the marine laboratory in Millport, to whom I am grateful for material.

The packing volume (as described in the previous section of this introduction) of T. seucica was $8,53 \times 10^{-10} cm^3$. $Cell^{-1}$ (standard deviation = $1,55 \times 10^{-10}$).

CHAPTER 1

"The effects of inorganic particulate suspensions on the filtration and ingestion rates of the ascidians Ciona intestinalis (L) and Ascidiella scabra (Müller), with particular reference to suspension density".

INTRODUCTION

Many investigators have calculated discrete rates of filtration for ascidians (Hecht, 1916; Jørgensen, 1949; Carlisle, 1966 and Fiala-Médioni, 1973). To talk of filtration rate is, however, to talk of an abstract, since innumerable factors influence the process continually and to differing degrees (Morton, 1971).

Rates have been calculated as functions of body size, species, temperature, oxygen tension and water movement (Randløv and Riisgård, 1979; Fiala-Médioni, 1974, 1978c, 1979b and Holmes, 1973). There are, however, few data concerning the effects of particulate concentration. Fiala-Médioni (1979a) reported a reduction in filtration rate at high algal cell concentrations. Holmes (1973), on the other hand, found no change in filtration rate. There have been no investigations involving inorganic particulates.

The effects of inorganic particulate suspensions have been studied in bivalve molluscs (Chiba and Ohshima, 1957; Loosanoff, 1962; Morton, 1971; Foster-Smith, 1975b and Theisen, 1977), sponges (Gerrodette and Fleshig, 1979) and the slipper limpet (Johnson, 1972). The results are contradictory; some authors reporting a reduced filtration rate with increasing particle concentration, others reporting no change.

Phytoplankton concentrations have been found, in most cases, to have no effect on the feeding of bivalve molluscs. Filtration rate is reported to be independent of food availability in Pecten irradians (Chipman and Hopkins, 1954), Lasaea rubra (Ballantine and Morton, 1956) and Mytilus edulis (Jørgensen, 1952, 1960 and Thompson and Bayne, 1974).

More recent investigations have, however, revealed that filtration rate in lamellibranchs can be adjusted to maintain a constant ingestion rate (see reviews by Winter, 1977, 1978).

Since Rigler (1961) discovered an upper limit to the rate of ingestion of Daphnia spp. and attributed it to an intrinsic feature of zooplankton behaviour, a series of mathematical models have been proposed to predict the behaviour of filter-feeders at changing particulate concentrations (Lehman, 1976; Lam and Frost, 1976). The basis of these models is the assumption that filter-feeders tend to maximise their nettrate of energy intake (optimal foraging theory). The models predict that, once the gut is tightly packed, the rate of filtration will decrease as the particulate suspension becomes increasingly dense. Winter (1978) proposed a similar model for filter-feeding in bivalve molluscs.

Data concerning the feeding of zooplankton fit the models very well, but data for filter-feeding bivalves are more contradictory.

It was hoped in this study to ascertain the effects of increasing inorganic particulate concentrations on the filtration and ingestion rates of the ascidians Ciona intestinalis and Ascidiella scabra. A. scabra (mud) and A. scabra (Fucus) (see General Introduction) have been dealt with separately.

MATERIAL AND METHODS

Filtration rates (defined as volume cleared of suspension) were calculated by measuring the rate of removal of inorganic particulate suspensions with an absorptiometer (Corning-EEL), utilizing a neutral density filter and 10,5cm pathlength cells, with seawater filtered to 0,3µm (Whatman Gamma 12, in-line filter system) used as a blank. The absorptiometer was calibrated against suspensions of inorganic particulates in filtered seawater. One litre samples of the same suspensions were filtered on preweighed Whatman GFC glass microfibre filters. The filter papers were washed twice with distilled water, dried for 24 hours at 105°C, and the weight gain attributed to the inorganic particulate load. The calibration curves obeyed Beer's law.

Ascidians were placed in 3 litre jars, maintained at 15°C in a waterbath. One ascidian was placed in each jar, with the exception of the smaller A. scabra (Fucus) where 5-7 ascidians were added to each jar. The animals were allowed to acclimatize for 24 hours before each experiment. A control jar with no animals was included in each experiment.

Suspensions of inorganic particulates were added 30 minutes before each experiment and were kept in suspension by an airstone in each jar. Each experiment was run for 1 hour, the particulate load being measured at 0, 30 and 60 minutes. The results at 30 minutes were used as mean suspension loads.

Filtration rates were calculated using the formula of Quayle (1948) as suggested by Coughlan (1969). A correction factor was added to account for natural settlement of particles, as derived by Willemsen (1952). A further correction factor was also included to account for

the reduction in water volume due to sampling. The final equation was thus:

$$\frac{df}{dt} = \left[\frac{V_o + V_t}{2\ n\ t}\right]\left[\log_n\left(\frac{S^e_o}{S^e_t}\right) - \log_n\left(\frac{S^c_o}{S^c_t}\right)\right]$$

where: $\frac{df}{dt}$ = filtration rate

V_o = volume of water in experimental jar after withdrawal of sample at time o.

V_t = volume of water in experimental jar before withdrawal of sample at time t.

n = number of animals.

t = duration of experiment.

S^e_o, S^e_t = suspension loads in experimental jar at times o and t.

S^c_o, S^c_t = suspension loads in control jar at times o and t.

After experimentation, animals were allowed to void their gut contents in filtered sea water for 48 hours. The animals were then rinsed in distilled water, dried for 24 hours at 105^{o}C and weighed. Evacuation was found to be extremely important for animals filtering inorganic material, as the gut contents greatly increased the animals' dry weights.

From these data, curves of best fit were plotted, using running means. Ingestion rates were calculated as the product of filtration rate and suspension load, as suggested by Winter (1978). This is possible as pseudofaeces are not produced by ascidians (Fiala-Médioni, 1974).

	C. intestinalis		A. scabra (mud)		A. scabra (Fucus)	
	ORGAN	TOTAL	ORGAN	TOTAL	ORGAN	TOTAL
Fuller's earth	87,2 ± 76,3	173,4 ± 124,1	92,1 ± 9,6	220,4 ± 39,3	17,9 ± 5,0	32,1 ± 7,2
Kaolin	80,8 ± 57,1	189,4 ± 98,1	101,9 ± 31,9	233,7 ± 42,6	17,9 ± 5,0	32,1 ± 7,2
Ballochmartin mud	80,8 ± 57,1	189,4 ± 98,1	101,9 ± 31,9	233,7 ± 42,6	15,9 ± 3,6	29,4 ± 6,0

TABLE 3 Dry weights (mg) and 95% confidence limits of ascidians used in filtration experiments.

RESULTS

Filtration rates at low particulate suspension loads were of the order of 0,7 $l.h^{-1}$ for C. intestinalis, 0,2 $l.h^{-1}$ for A. scabra (Mud) and 0,1 $l.h^{-1}$ for A. scabra (Fucus). These rates were consistent for all three particulate types studied.

Filtration rates decreased, with increasing suspension loads, in all cases (Figs. 6-8). The decreases were all significant ($p < 0,001$; Kendall's coefficient of rank correlation, Table 4). The decrease was most acute in C. intestinalis and least acute in A. scabra (Fucus). The rates of ingestion, however, show that this is just an effect of scale.

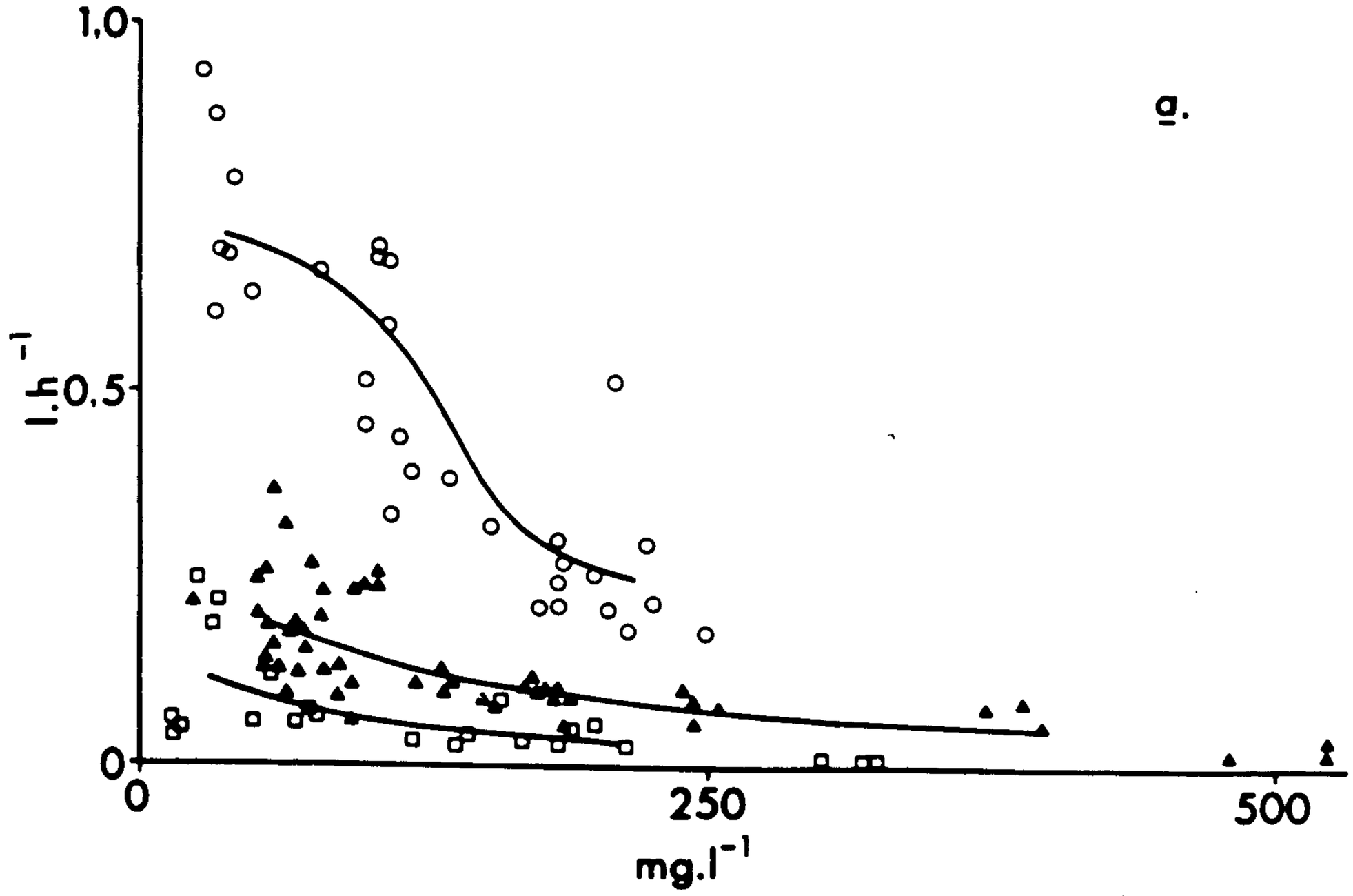
In all cases, ingestion rates reach a virtually constant level after an increase. The satiation point is indistinct due to the effects of running means and the inaccuracy of the method at lower suspension loads.

The volumes ingested per hour at the maximum ingestion rates (Table 5) were calculated using the packed particle volumes of the inorganic particulates (General Introduction, Table 2). These rates are not consistent between the different particulates. The ratio of maximum volumetric ingestion rates between the three ascidian types studied are consistent between the different particulates used (Table 6). Weight-specific maximum volumetric ingestion rates (Table 7) are similar for C. intestinalis and A. scabra (Fucus), but halved in A. scabra (Mud).

FIGURE 6

a) Filtration rates ($l.h^{-1}$) of C. intestinalis (○), A. scabra (mud) (▲) and A. scabra (Fucus) (□) exposed to varying suspension loads ($mg.l^{-1}$) of Fuller's earth.

b) Ingestion rates ($mg.h^{-1}$) of C. intestinalis (C), A. scabra (mud) (AM) and A. scabra (Fucus) (AF) exposed to varying suspension loads ($mg.l^{-1}$) of Fuller's earth.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0,5
0
0
250
500
$mg.l^{-1}$
$mg.l^{-1}$
b.
60
C
AM
AF
$mg.h^{-1}$
0
0
250
500
$mg.l^{-1}$

FIGURE 7

a) Filtration rates ($l.h^{-1}$) of C. intestinalis (○), A. scabra (mud) (▲) and A. scabra (Fucus) (□) exposed to varying suspension loads ($mg.l^{-1}$) of Kaolin.

b) Ingestion rates ($mg.h^{-1}$) of C. intestinalis (C), A. scabra (mud) (AM) and A. scabra (Fucus) (AF) exposed to varying suspension loads ($mg.l^{-1}$) of Kaolin.

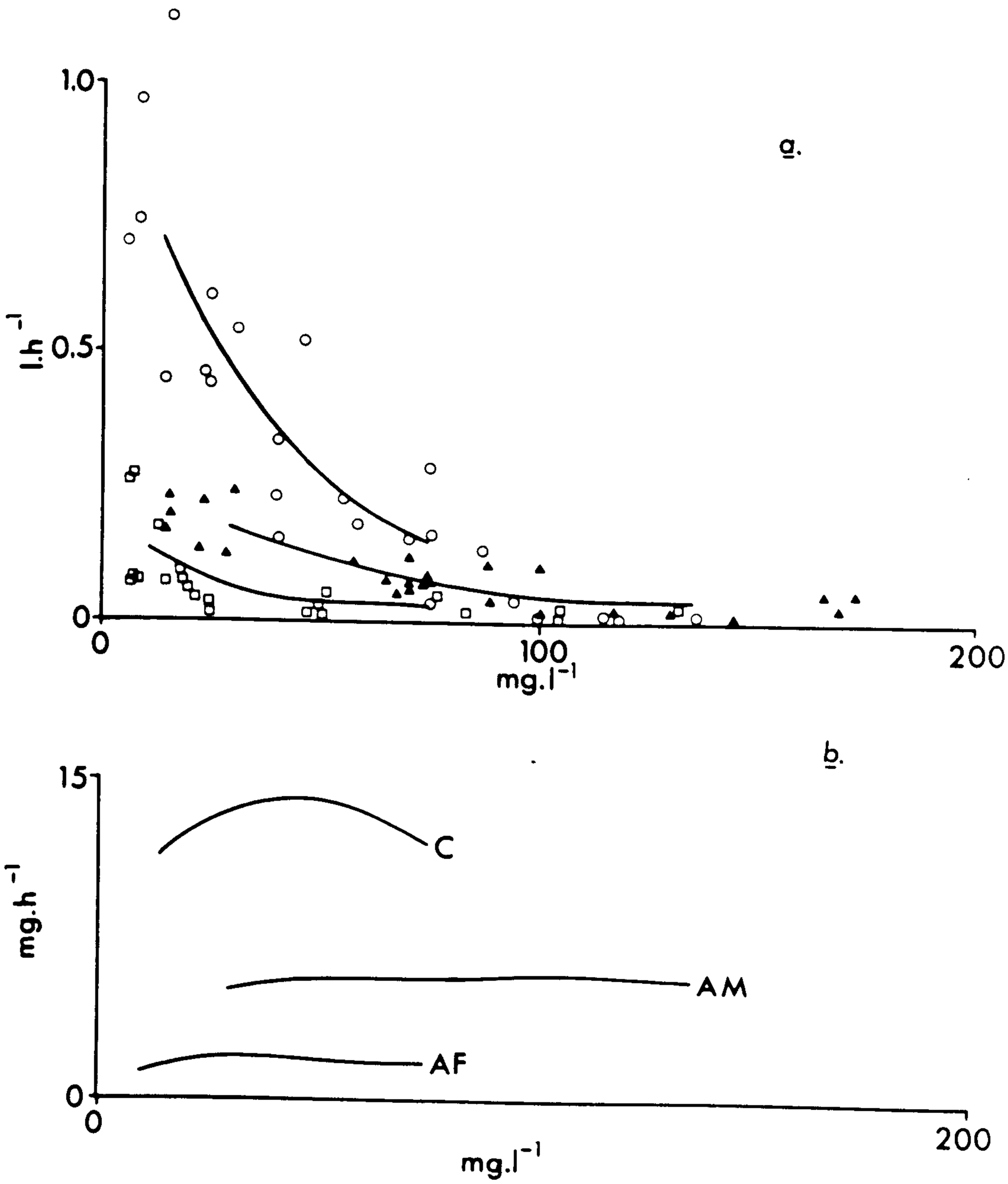
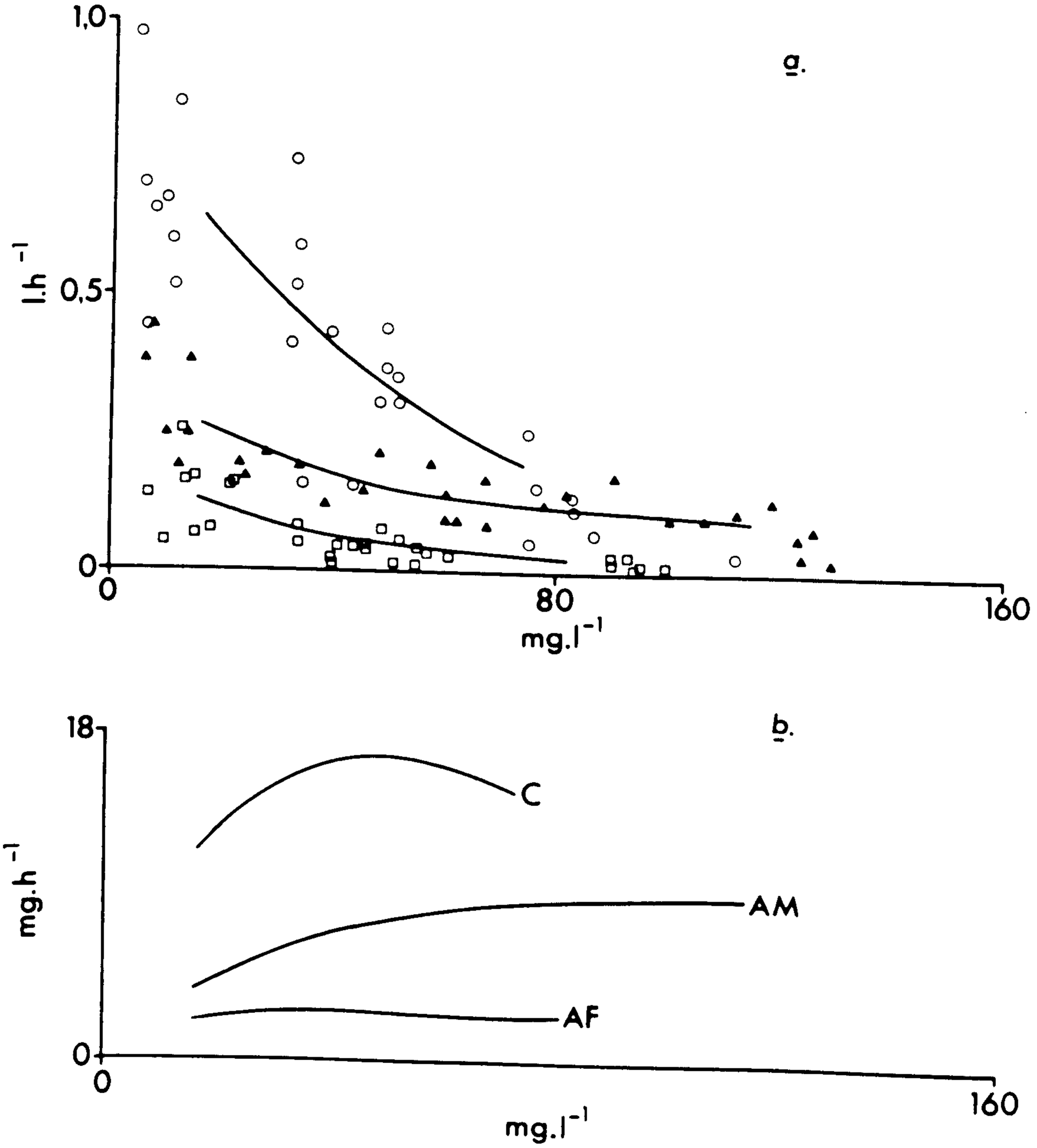

a.
1.0
0.5
0
0
100
200
mg.l-1
h-1
b.
15
0
0
200
C
AM
AF
mg.l-1
mg.h-1

FIGURE 8

a) Filtration rates ($l.h^{-1}$) of C. intestinalis (○), A. scabra (mud (▲) and A. scabra (Fucus) (□) exposed to varying suspension loads ($mg.l^{-1}$) of Ballochmartin mud.

b) Ingestion rates ($mg.h^{-1}$) of C. intestinalis (C), A. scabra (mud) (AM) and A. scabra (Fucus) (AF) exposed to varying suspension loads ($mg.l^{-1}$) of Ballochmartin mud.


a.
1,0
0,5
0
0
80
160
mg.l⁻¹
l.h⁻¹
b.
18
0
0
160
mg.l⁻¹
mg.h⁻¹
C
AM
AF

ANIMAL	PARTICULATE	S	τ	d	SIGNIFICANCE
	Fuller's earth	-340	-0,74	-5,78	0,001
C. intestinalis	Kaolin	-261	-0,76	-5,44	0,001
	Ballochmartin mud	-299	-0,75	-5,61	0,001
	Fuller's earth	-810	-0,59	-6,21	0,001
A. scabra (mud)	Kaolin	-200	-0,62	-4,41	0,001
	Ballochmartin mud	-294	-0,68	-5,25	0,001
	Fuller's earth	-131	-0,52	-3,46	0,001
A. scabra (Fucus)	Kaolin	-136	-0,60	-3,83	0,001
	Ballochmartin mud	-287	-0,63	-4,88	0,001

TABLE 4 Kendall's coefficients of rank correlation for filtration data.

	Fuller's earth	Kaolin	Ballochmartin mud
C. intestinalis	176	672	37
A. scabra (mud)	62	292	21
A. scabra (Fucus)	19	98	6

TABLE 5 Maximum volumetric ingestion rates ($\mu l.h^{-1}$) of C. intestinalis and A. scabra exposed to three inorganic suspension types.

Fuller's earth	9,3	:	3,3	:	1,0
Kaolin	6,9	:	3,0	:	1,0
Ballochmartin mud	6,2	:	3,5	:	1,0

TABLE 6 Ratios of volumetric maximum ingestion rate C. intestinalis : A. scabra (mud) : A. scabra (Fucus).

	Fuller's earth	Kaolin	Ballochmartin mud
C. intestinalis	2,02	8,32	0,46
A. scabra (mud)	0,67	2,87	0,21
A. scabra (Fucus)	1,06	5,47	0,38

TABLE 7 Weight-specific maximum volumetric ingestion rates ($\mu l.h^{-1}.mg^{-1}$ organ dry weight) of C. intestinalis and A. scabra exposed to three inorganic suspension types.

DISCUSSION

The filtration rates of C. intestinalis at lower suspension loads are broadly consistent with rates calculated by previous authors, 0,5-0,7 $l.h^{-1}$ (Jørgensen, 1949) and 0,8-1,1 l,h^{-1} (Fiala-Médioni, 1978b). Filtration rates calculated for other ascidians have been somewhat higher. Rates of 0,83-5,10 (Carlisle, 1966) and 1,0-3,0 $l.h^{-1}$ (Fiala-Médioni, 1973) have been calculated for Phallusia mammillata, and a rate of 1,2-8,4 $l.h^{-1}$ determined for Ascidia atra (Hecht, 1916). Holmes (1973) calculated rates of 1,0-5,8 $l.h^{-1}$ for Styela clava and 0,8-3,2 $l.h^{-1}$ for Ascidiella aspersa. The lower rates found (above) for A. scabra are thought to reflect it's smaller size.

Decreasing filtration rates, at high particulate concentration, have been reported for Phallusia mammillata by Fiala-Médioni (1979a) using the unicellular alga Monochrysis lutheri between the concentrations of 2.10^{7} and 2.10^{8} cells l^{-1}. Monochrysis lutheri has a volume about one-eighth that of T. seucica and it's packing volume is likely to be approximately one-eighth that of T. seucica, the two being of similar shape. Using the packing volumes of T. seucica and Fuller's earth (General Introduction, Table 2), the cell concentrations reported by Fiala-Médioni are equivalent to 0,6-6,3 $mg.l^{-1}$ Fuller's earth. The filtration rate in her experiments, thus, starts declining at a similar suspension load to that found in the present investigation.

Jørgensen (1954), however, reported that the filtration rate of ascidians is independent of the concentration of graphite particles in water. It is possible, though, that he did not use a sufficiently high concentration to induce a reduction in filtration rate.

Filtration rates of other filter-feeding animals have been more extensively studied. Loosanoff (1962) found reductions in the pumping

rates (to which filtration rates are proportional) of oysters, using various inorganic particulate suspensions. Comparing his results with the early work of Loosanoff and Engle (1947), he concluded that pumping rate in oysters is reduced by increased turbidity, regardless of whether this turbidity is caused by large numbers of micro-organisms or by inorganic particulate suspension.

Results from other investigations with filter-feeding bivalve molluscs are contradictory. Decreases in filtration rate at high particulate concentration have been reported by several authors (Davids, 1964; Winter, 1969, 1970, 1973; Ali, 1970; Morton, 1971; Walne, 1972; Foster-Smith, 1975a,b; Schulte, 1975; Theisen, 1977; Widdows et al., 1979; Kiørboe et al., 1980).

Several other authors, however, have reported filtration rate to be independent of particulate concentration (Chiba and Ohshima,1957; Jørgensen, 1952, 1960; Chipman and Hopkins, 1954; Ballantine and Morton, 1956; Thompson and Bayne, 1974; Riisgård and Møhlenberg, 1979). If the models of Lam and Frost (1976) and Winter (1978) are correct, however, there will be a wide range of particulate concentrations at which the filtration rate will remain unchanged. The filtration curves, reported by Johnson (1972), for the slipper limpet, Crepidula fornicata, exposed to 100-1600 mg./l. silt, are very like those of ascidians reported here.

Harbison and Gilmer (1976) report no change in the filtration rate of salps, but for the copepods Calanus spp (Corner et al., 1972; Frost, 1972) and the cladocerans Daphnia spp (Burns and Rigler, 1967) filtration curves are similar to those of ascidians.

Clay, at suspension loads of 2,8-95,4 $mg.l^{-1}$, has been shown to reduce the pumping rate of the sponge Verongia lacunosa (Gerrodette and Fleschig, 1979). Excessive concentration of the unicellular alga Phaeodactylum reduces the filtration rate of the anostracan Artemia, although at moderate concentrations the filtration rate is constant

(Reeve, 1963a). Downing and Peters (1980) concluded that filtration rate can be predicted from body length and suspension concentration in the cladoceran Sida crystallina.

The reduction in filtration rates of ascidians is such that the rate of ingestion remains constant (Figs. 6-8). This fulfills the optimal foraging model predictions of Lehman (1976) and Lam and Frost (1976) who suggest that, at an ingestion rate at which the gut is completely packed, the filtration rate will start to decrease. Hughes (1980) reviewed these models and concluded that the decline in optimal filtering rate, at particle densities above the satiation point, occurs because the gut can be fully packed at progressively lower filtering rates. Once the gut becomes full, high filtering rate would merely cause a 'bottle neck' of food and incur higher filtering costs with little or no extra gain in energy intake.

Similar levelling-off of ingestion rates has been reported for Artemia (Reeve, 1963a), Daphnia (Burns and Rigler, 1967; McMahon, 1965; McMahon and Rigler, 1963, 1965; Rigler, 1961), Bryozoa (Bullivant, 1968) and copepods (Corner et al., 1972; Frost, 1972; Marshall and Orr, 1955).

Data for bivalve molluscs are once again more contradictory. Foster-Smith (1975a) has shown that the ingestion rate of Mytilus edulis levels off, but that those of Cerastoderma edule and Venerupis pullastra reach a maximum and then descend. Levelling-off was found in mussels and oysters by Schulte (1975), Tenore and Dunstan (1973) and Widdows et al., (1979). Tenore and Dunstan, however, found a slight decrease in the ingestion rate of clams at increased particulate densities. The issue is complicated in bivalves by their ability to produce pseudofaeces.

Nevertheless, filter-feeders generally reach and maintain a maximum ingestion rate as predicted by Rigler (1961).

Morton (1971) and Frost (1972, 1975) have remarked that an increase

in the size of the algal food cells results in a decrease in the cell numbers required to elicit the maximum filtration rate, suggesting that particle volume is more important than simply particle number. McMahon and Rigler (1965) found a greater similarity of maximum ingestion rates, of various foods, when expressed volumetrically. Reeve (1963b), however, found that 12X the volume of sand was ingested as compared with algal cells by Artemia, but admitted the difficulty involved in measuring the volume of the sand grains.

Considering the consistency of the ratios of maximum volumetric ingestion rates between C. intestinalis and A. scabra (Fig. 7), the inconsistency of the maximum ingestion volumes (Fig. 6) is most likely caused by inaccuracy in the method of volumetric measurement. This is possibly due to a difference in packing force. The packing force in the experiments on particle packing volume was gravitational and hence proportional to the weight of the particles. The packing force in the ascidian filter-feeding mechanism is, however, largely independent of particle weight.

Although Theisen (1977) has discovered that different morphological forms of Mytilus edulis are related to turbidity levels, the difference in morphology of A. scabra from mud and Fucus is not considered to be analagous. The responses of both morphs to increased inorganic particulate load were similar, although the weight-specific ingestion rate was greater for animals from Fucus (Table 7). This is thought to be an effect of size, with smaller animals filtering proportionately more food than large ones (Widdows, 1978b).

CHAPTER 2

"The effects of body-size and temperature on the filtration and ingestion of inorganic particulate suspensions by the ascidian *Ciona intestinalis* (L.)

INTRODUCTION

A basic feeding curve was established for ascidians in Chapter 1, which showed similarities with the models of filter-feeding proposed for copepods (Lam and Frost, 1976) and lamellibranch molluscs (Winter, 1978). Lam and Frost predicted the effects of body-size in their model, and Winter reports that large and small individuals of Mytilus edulis react in a similar manner to different algal concentrations. Temperature, however, was not considered in either model.

Body-size sets the absolute limit of metabolic activity and is referred to here as a metabolic determinator. Temperature, on the other hand, increases or reduces the metabolic rate within the limits set by body-size and is here termed a metabolic modifier.

Despite a general lack of consideration of particulate suspension density, there is a large body of data concerning the functional relationships of filtration rate with temperature and body size.

Filtration rate is generally expressed as a function of body weight by the allometric equation:

$$F = cW^b \qquad (1)$$

where F = filtration rate, W = body weight and c, b are constants. c is a function of the particular Y-axis scale chosen, b, however, is not affected by scale and is used by most investigators to describe the relationship. In the following experiments, F is replaced by Fmax.

The gradient (a) between any two data points may be calculated from the equation:

$$\frac{Fmax_1}{Fmax_2} = \frac{W_1}{W_2}^{a_f} \qquad (2)$$

(see appendix for derivation).

An average ($\bar{a}_f$) may be calculated for the values of a, between one data point and all the others. Values of $\bar{a}_f$ were accordingly calculated for each weight group of C. intestinalis. If the value b, calculated from equation 1, describes accurately the relationship between Fmax and dry organ weight, the values of $\bar{a}_f$ plotted against W should produce a straight line with a regression coefficient of zero. $\bar{a}_f$ can, hence, be used to test the fit of the results to the allometric equation calculated to describe them.

Equation 2 resembles the equation given by Lam and Frost (1976):

$$\frac{Imax_1}{Imax_2} = \left(\frac{L_1}{L_2}\right)^3 \qquad (3)$$

where Imax = maximum ingestion rate; L = length. Assuming that weight is a cubic function of length (i.e. $W \propto L^3$) the equation can be modified to

$$\frac{Imax_1}{Imax_2} = \left(\frac{W_1}{W_2}\right)^{a_I} \qquad (4)$$

where a_I is equal to one. Values of $\bar{a}_I$ for ingestion rate can, hence, be calculated from equation 4 as they were for filtration rate from equation 2.

Temperature has been considered classically as one of the major environmental modifiers of the rate of metabolism and level of activity of poikilothermic organisms (Bayne et al., 1976). The changes in activity are normally described by the factor by which they are changed over a ten degree temperature change, Q_{10}.

The relationship between temperature and activity is altered by acclimation. Kinne (1963) split the responses of poikilotherms to

temperature change on a temporal basis. First there is an immediate response lasting, at most, a few minutes, in which there may be an over- or an under-shoot of activity. This is followed by a period of stabilization, lasting several hours. Finally there is acclimation to a new steady state. It is from the stabilized rates that Q_{10} determinations can be made (Prosser and Brown, 1973).

The effects of these two factors, viz. temperature and body weight, on the filtration and ingestion of suspensions of Fuller's earth by C. intestinalis have been investigated. Dry organ weight (i.e. minustest) was used as a measure of body-size. Coughlan and Ansell (1974) have criticised the use of dry organ weight as a measure of body-size, since it is affected by the amount of storage products present and the stage of development of the gonads which may, or may not, affect pumping activity. Walne (1972), however, still regards it as the best measure, and Fiala-Médioni (1973) found a good fit of data for filtration rate against dry organ weight in Phallusia mammillata.

MATERIAL AND METHODS

For methods of calculation of filtration rates, ingestion rates and lines of best fit see Chapter 1. Procedural details were as follows.

i. Body-size experiments

C. intestinalis of 3 size groups were placed in separate 3-litre jars in a 15°C waterbath. The number of individual animals in each jar was adjusted, according to size, such that the overall filtration rates for the jars were approximately equal.

ii. Temperature experiments

Medium-sized *C. intestinalis* were placed in separate 3-litre jars in a 15°C waterbath. The animals were allowed to acclimate to this temperature for several days. The temperature of the waterbath was adjusted to the required temperature (5°C, 10°C or 15°C) five hours before each experiment.

The water samples taken to measure the suspended particulate load were kept in sealed bottles until they reached room temperature, since cold samples caused condensation on the glass faces of the absorptiometer cuvettes.

Q_{10} values were calculated using the formula:

$$\log Q_{10} = \frac{10\ (\log k_1 - \log k_2)}{t_1 - t_2}$$

where k = velocity constant (in this case Fmax)
t = temperature.

	ORGAN	TOTAL
Large	61,4 ± 12,9	351,0 ± 122,6
Medium	16,4 ± 8,5	67,4 ± 18,7
Small	1,5 ± 0,6	5,9 ± 1,9

TABLE 8a Dry weights (mg) and 95% confidence limits for ascidians used in filtration experiments (body-size).

	ORGAN	TOTAL
Medium	23,3 ± 5,5	113,3 ± 26,7

TABLE 8b Dry weights (mg) and 95% confidence limits for ascidians used in filtration experiments (temperature).

RESULTS

a) The effects of body-size on the filtration and ingestion rates

Filtration rate decreased with increasing particulate concentration for all three body-size groups investigated (Fig. 9a). The decrease was significant in all cases ($P < 0,001$, Kendall's coefficients of rank correlation; Table 9).

Ingestion rates remained constant (Fig. 9b). The filtration and ingestion rates were highest for the largest animals and lowest for the smallest.

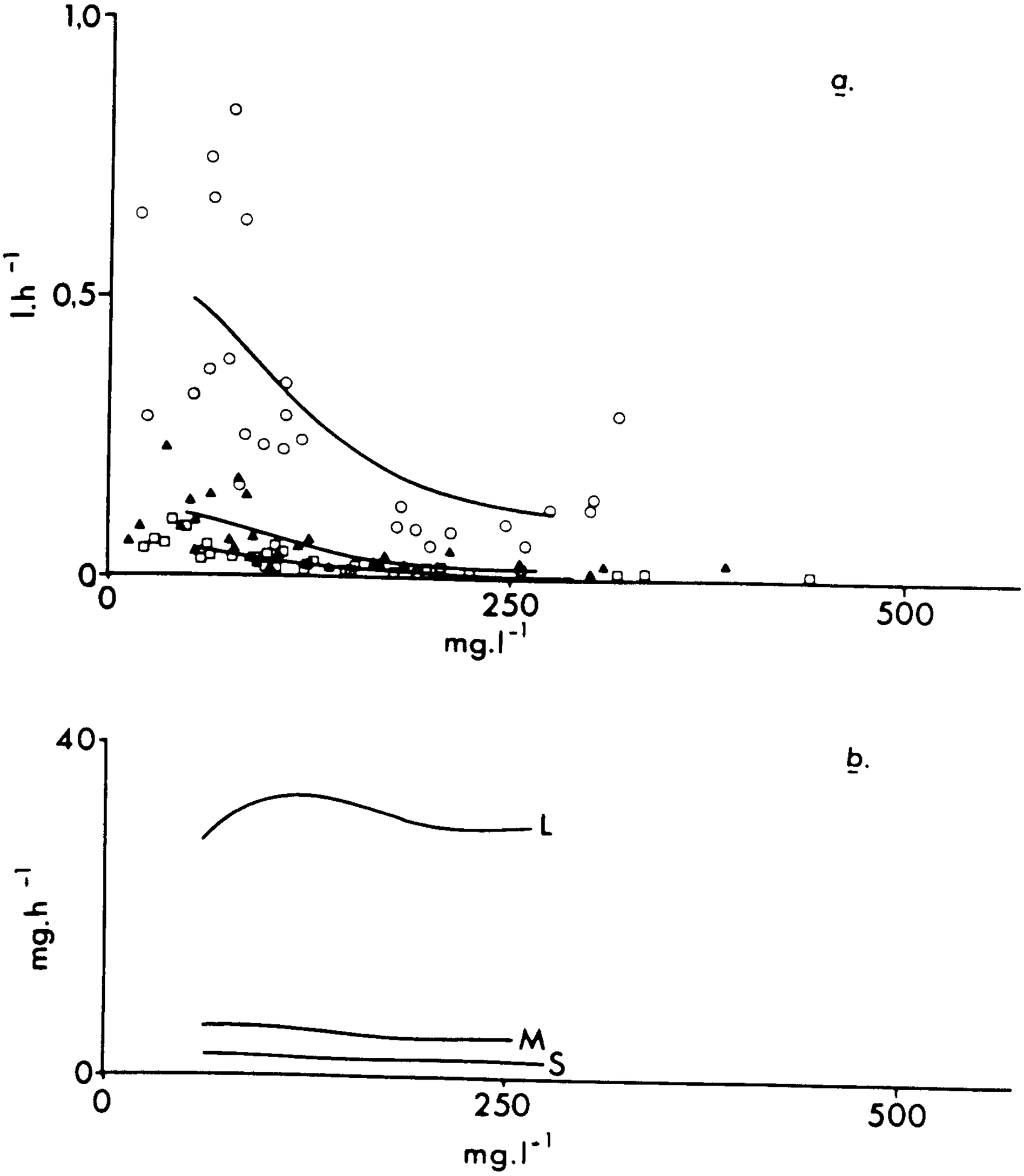
Maximum filtration rate (estimated from Fig. 9a) is linearly related to dry organ weight when data are log transformed (Fig. 10, including also results for C. intestinalis from Chapter 1 and the 15°C temperature experiments in Chapter 2). Regression lines were calculated, by the method of least squares, for all results and for all results with the exception of the smallest size group. The allometric equations describing these regressions are $F = 40W^{0,65}$ for all results and $F = 19,4W^{0,83}$ for results excluding the smallest size group. The regression line for C. intestinalis calculated by Randlóv and Riisgård (1979) is also included in Fig. 2 for comparison. Values of $\bar{a}_f$ (Fig. 11a) produce a horizontal straight line with the exception of the value for the smallest size group. Values of $\bar{a}_I$ (Fig. 11b) do not produce a horizontal straight line, indicating that an allometric equation cannot properly be fitted to this function.

Weight specific maximum ingestion rates, calculated from Fig. 9b and Table 8a, are presented in Table 10. The rate is highest for the smaller animals.

FIGURE 9

a) Filtration rates ($l.h^{-1}$) of C. intestinalis exposed to suspensions of Fuller's earth ($mg.l^{-1}$). Large (○), medium (▲) and small (□) animals.

b) Ingestion rates ($mg.h^{-1}$) of C. intestinalis exposed to suspensions of Fuller's earth ($mg.l^{-1}$). Large (L), medium (M), small (S).


a.
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0
0
250
500
mg.l-1
l.h-1
b.
40
0
0
250
500
L
M
S
mg.h-1
mg.l-1

SIZE	S	τ	d	SIGNIFICANCE
Small	-260	-0,61	-4,64	0,001
Medium	-198	-0,49	-3,71	0,001
Large	-180	-0,52	-3,75	0,001

TABLE 9 Kendall's coefficients of rank correlation for filtration data (body-size).

FIGURE 10 Filtration rates of C. intestinalis of various sizes (● - Chapter 2, body size; ☆ - Chapter 2, temperature (15°C); ○ - Chapter 1, Fuller's earth, Kaolin and Ballochmartin mud). Solid lines are regressions as described in the text. Broken line is the regression calculated by Randlov and Riisgård (1979).

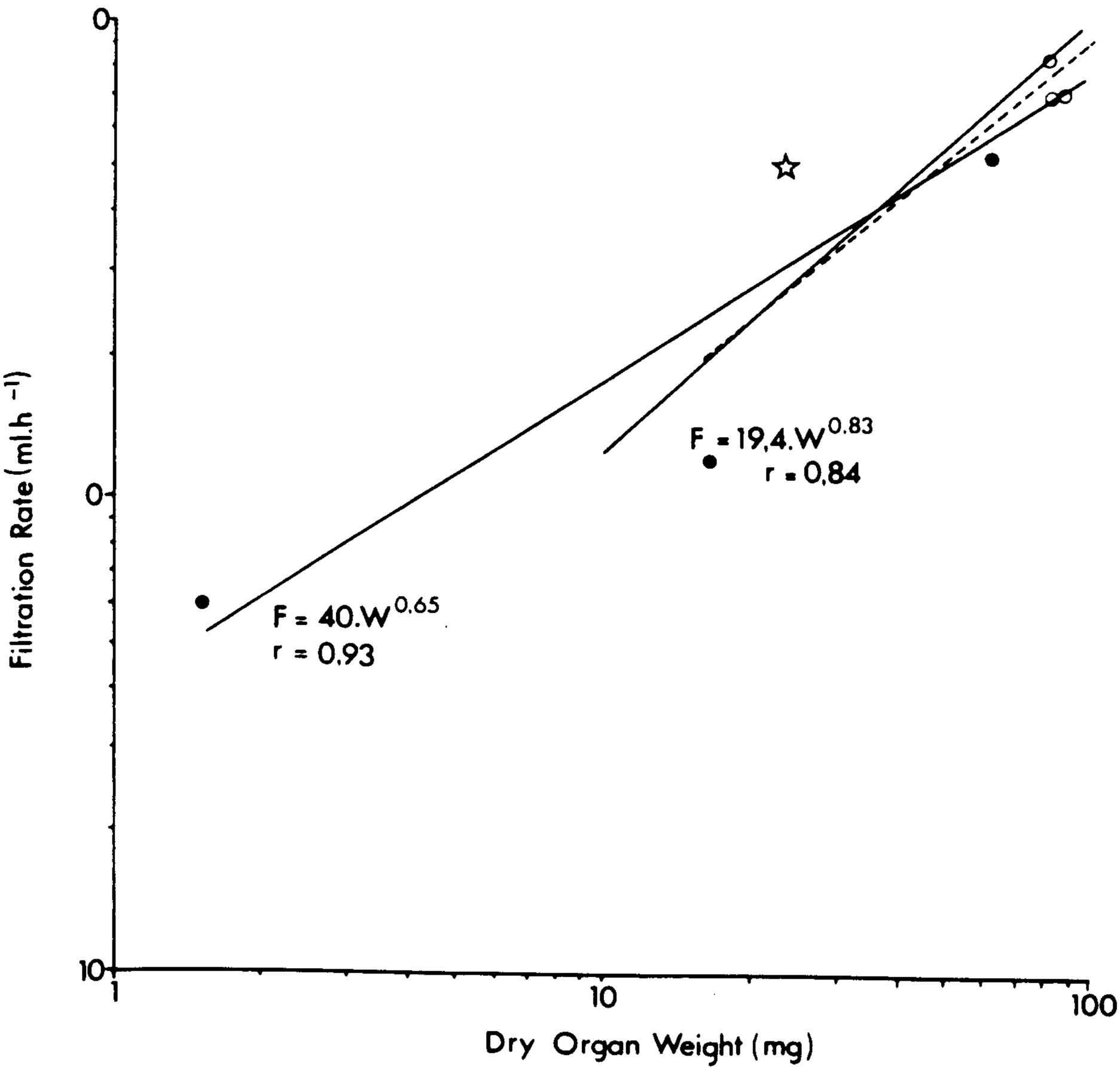
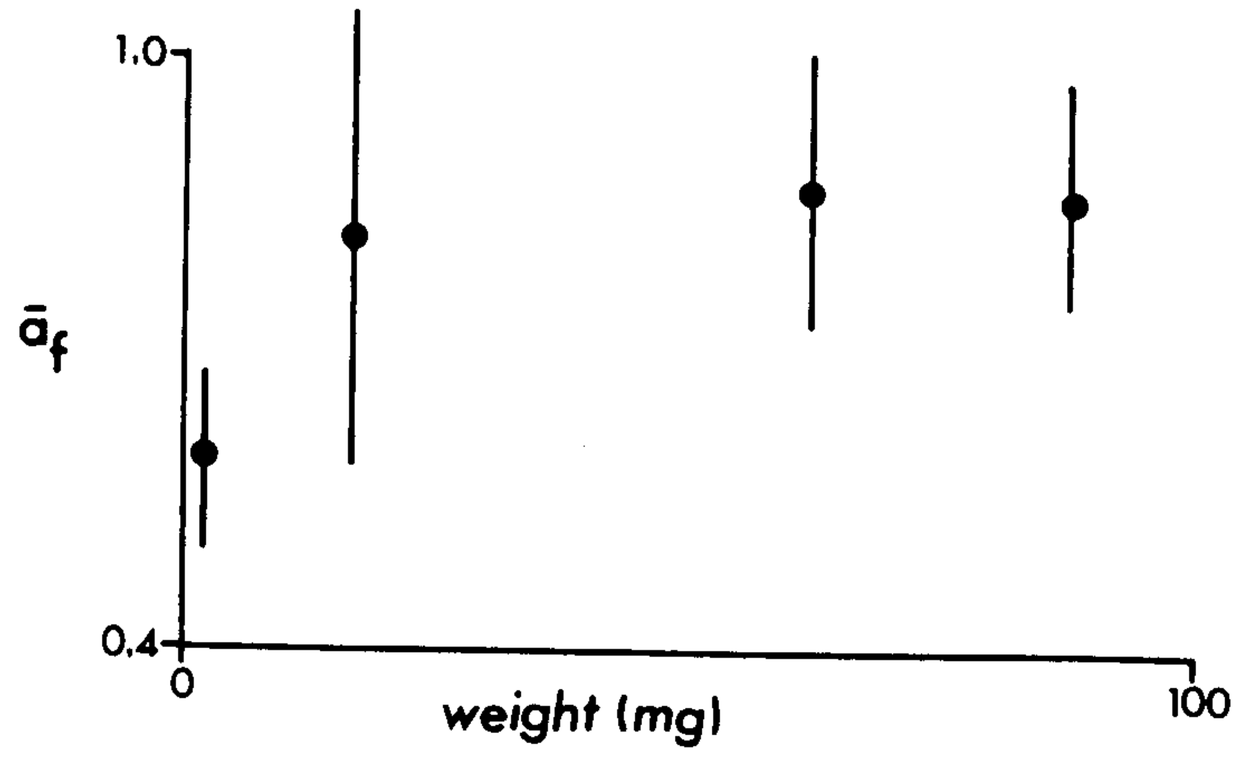

Filtration Rate (ml.h⁻¹)
0
0
10
1
10
100
Dry Organ Weight (mg)
F = 40.W^0.65
r = 0,93
F = 19,4.W^0.83
r = 0,84

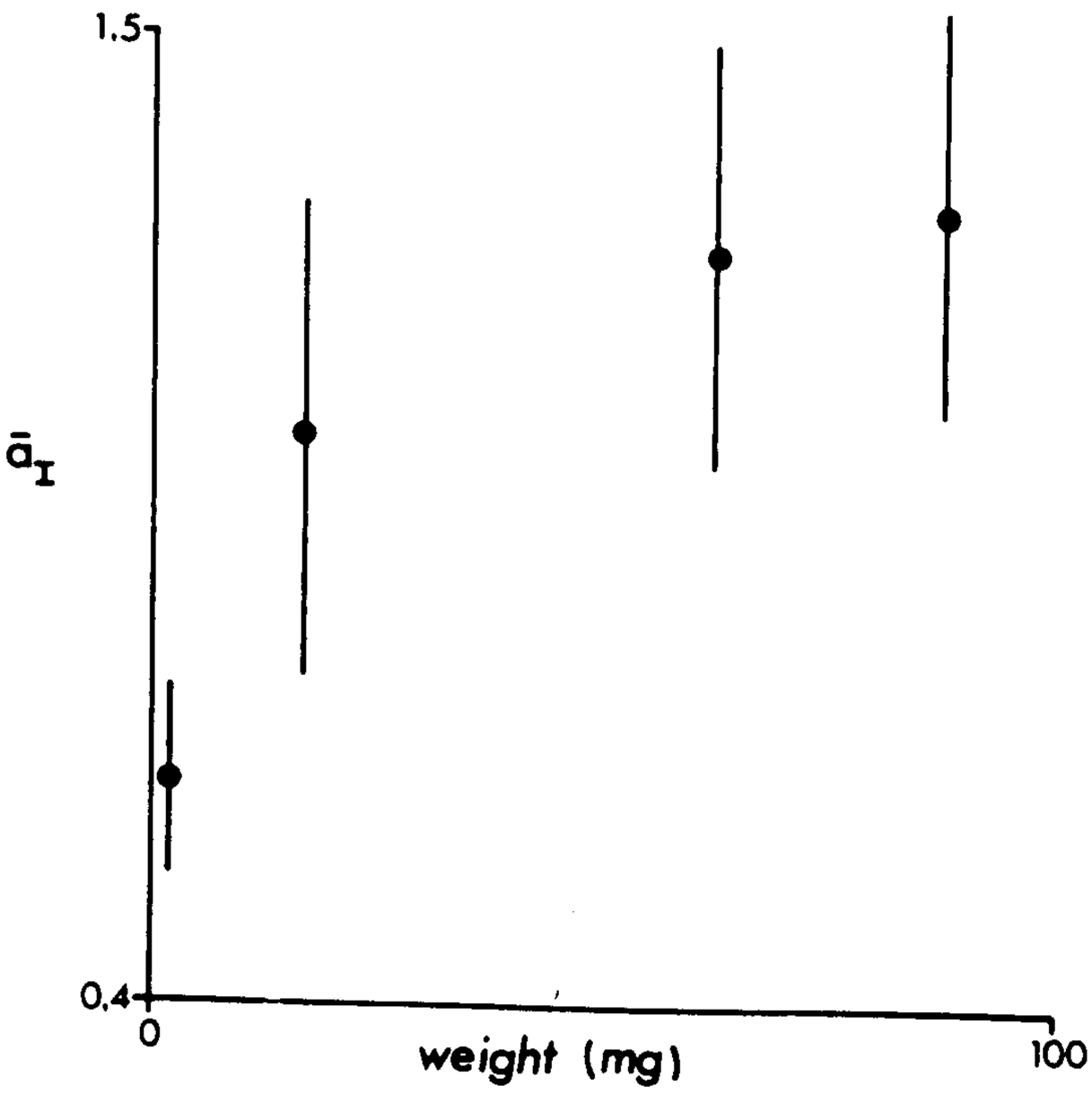
FIGURE 11 Values of $\bar{a}_f$ (a) and $\bar{a}_I$ (b).

Data includes results from Chapter 1, for C. intestinalis, filtering Fuller's earth.

a.



b.



SIZE GROUP	$\mu l.h^{-1}.mg^{-1}$ (DRY ORGAN WEIGHT)
Large	1,62
Medium	1,03
Small	4,20

TABLE 10 Weight-specific maximum ingestion rates of Ciona intestinalis.

TEMPERATURE RANGE	Q_{10} (Fmax)
5 - 10°C	4,00
5 - 15°C	4,20
10 - 15°C	4,34

TABLE 11 Q_{10} Fmax for C. intestinalis.

b) The effects of temperature on filtration and ingestion rates

At 15°C, the curve representing the decline in filtration rate is similar to those described in Chapter 1 and for the three body-size groups in this chapter. At the lower temperatures, however, the curves are only identical to that of 15°C at higher suspension densities. At lower particulate loads they level off. The filtration rate levels off at a lower suspension density at 10°C than at 5°C (Fig. 12a). These level portions are assumed to represent the maximum filtration rates of which the animals are capable at each temperature.

Ingestion rate increases to a maximum, this maximum being reached at progressively higher particulate loads with decreasing temperature (Fig. 12b).

Q_{10} values are all above 4 (Table 11) and, therefore, show a marked temperature dependence of filtration rate.

FIGURE 12

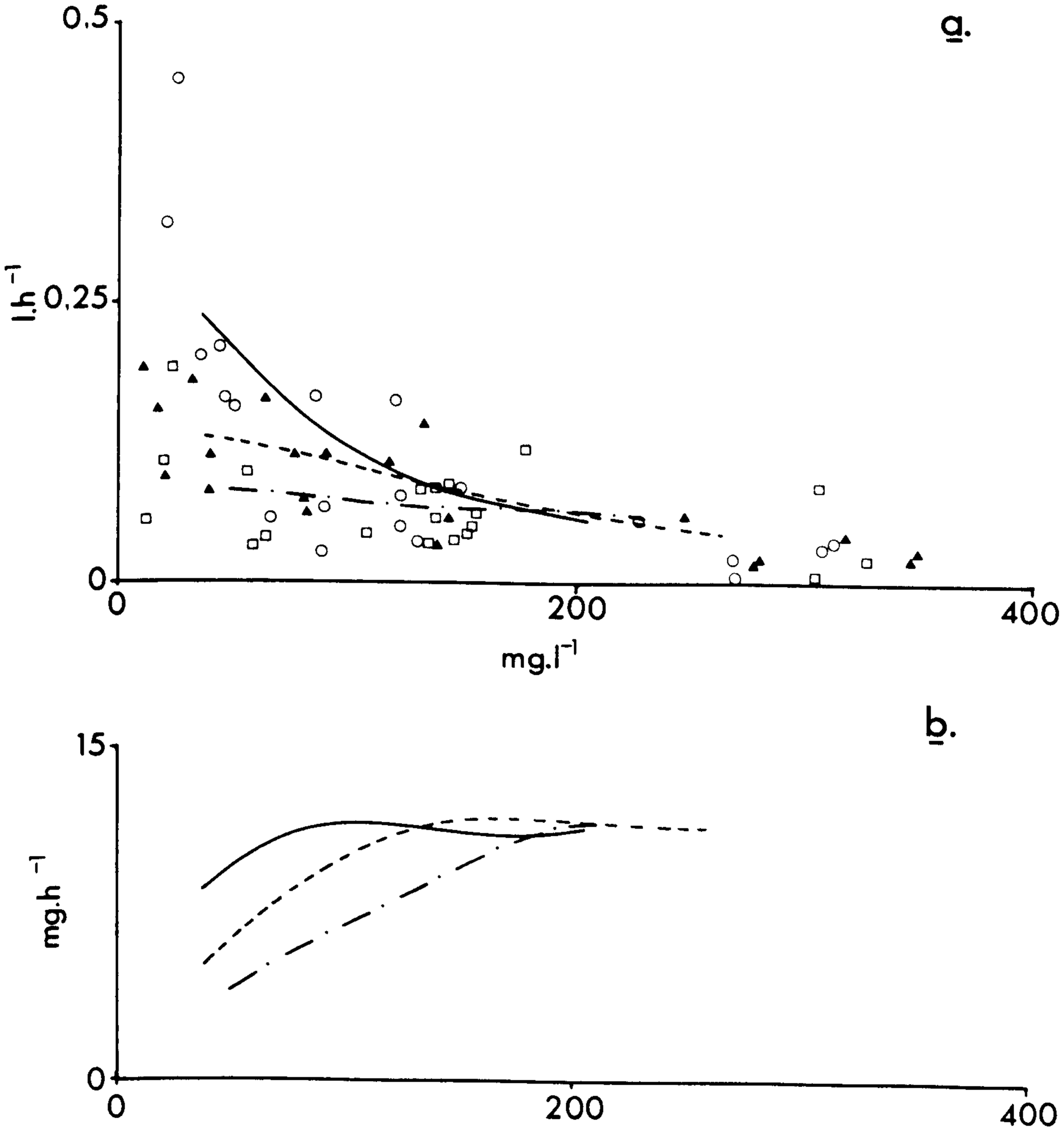
a) Filtration rates ($l.h^{-1}$) of C. intestinalis exposed to suspensions of Fuller's earth ($mg.l^{-1}$) at various temperatures.

b) Ingestion rates ($mg.h^{-1}$) of C. intestinalis exposed suspensions of Fuller's earth ($mg.l^{-1}$) at various temperatures.

○——————○ = 15°C

▲- - - - - - - -▲ = 10°C

□—·—·—□ = 5°C


a.
0.5
0.25
0
0
200
400
mg.l⁻¹
b.
15
0
0
200
400
mg.l⁻¹

DISCUSSION

A decrease in filtration rate with increasing particulate concentration, such that ingestion rates remain constant, is consistent with the results of Chapter 1.

The effects of body-size on filtration rate in bivalve molluscs is now well documented, and the results have been reviewed by several authors (Jørgensen 1966, 1975, 1976; Vahl, 1973b and Winter, 1978). Winter (1978) reported that large and small individuals of Mytilus edulis reacted in a similar manner to different algal concentrations, as would appear to be the case in C. intestinalis. Most authors have ignored the effects of algal concentration, however, and usually plot filtration rate against body weight regardless of experimental conditions.

It is generally accepted that filtration rate (and other functions determined by metabolic rate) increases with increasing body-size, in accordance with the general allometric equation $F = cW^b$. Also, smaller animals have a higher weight-specific metabolic rate than larger ones (Vernberg and Vernberg, 1969). The value of b is, hence, generally less than 1.

Furthermore, if filtration rate is assumed to be a function of surface area, as most filters tend to be flattened surfaces, (i.e. $F \propto L^2$) and weight to be a function of volume ($W \propto L^3$), the allometric relationship can be theoretically derived as $F \quad W^{\frac{2}{3}}$, i.e. b = 0,67. Hemmingsen (1950, 1960) has found that b (for standard metabolism) is nearer 0,75 for all organisms and in practice b is accepted as being between 0,66 and 1,00, with a general approximate mean of 0,75 (Jørgensen, 1976; Widdows, 1978a; Winter, 1978; Møhlenberg and Rissgard, 1979; Riisgard and Møhlenberg, 1979). Many authors, though, quote lower values of b for lamellibranch bivalves (Widdows, 1978a; Vahl, 1973a,b; Willemsen, 1952; Theede, 1963; Winter, 1973 and Thompson and

Bayne, 1974). Coughlan and Ansell (1964) suggested that b changes from 0,67 at a typical pumping rate to 0,701 at maximum pumping rates in Venus mercenaria. In ascidians, at least, only maximum rates are truly comparable as filtration rate decreases with increasing particulate load.

Values of b derived for ascidians are fewer. Randlóv and Riisgård (1979) calculated values of 0,91 for C. intestinalis and 0,88 for Ascidiella aspersa. These values are remarkably similar to the values found in the present study (Fig. 2). Hecht (1916) noted an increase in filtration rate with wet weight of Ascidia atra, as did Fiala-Médioni (1973) with the dry weight of Phallusia mammillata. I have calculated from Fiala-Médioni's results that b = 1,00. Holmes (1973) calculated b to lie between 0,617 and 0,646 for Styela clava and 0,383 - 0,607 for Ascidiella aspersa according to temperature. These results were calculated for water transport rate calculated by a direct method. Holmes found lower values of b when using a method involving suspension clearance (0,488 for S. clava and 0,275 for A. aspersa). The suspension density was high, in the present context, at 3×10^7 cells/litre. At this concentration the ascidians might be expected to have passed the satiation point and be in the declining portion of the filtration rate-versus-suspension density curve. At this concentration, therefore, the filtration rates are unlikely to be strictly comparable.

Values of b for ascidians would seem to be generally higher than those found for lamellibranch bivalves.

In addition to the decrease of weight specific filtration rate with increasing body-size, as shown by the values of b below 1 found by so many authors, Newell (1979) suggested that larger sized animals tend to have a rather lower rate of filtration than might be anticipated from the general allometric equation. Foster-Smith (1976) proposed that this could be due to a relative decrease in gill area in bivalves.

Similar findings for Mytilus edulis led Winter (1973) to conclude that the general allometric equation is an over-simplification; larger animals showing a more pronounced decrease in filtration rate. Values of $\bar{a}_f$ (Fig. 11a) would indicate that this is not the case for the larger size groups of C. intestinalis used in this study. These animals were not, however, equivalent to the largest specimens found in nature. The decrease in $\bar{a}_f$ for small individuals indicates that the allometric equation is inadequate for this size group. The decrease in b, when the smallest size group is included in the regression analysis (Fig. 10) would indicate that the smaller individuals show a precociously high filtration rate. So, for C. intestinalis, the general allometric equation appears to be an over-simplification at smaller sizes. Data points are few for this aspect of the study, however, and this statement is only tentative. Further research is clearly desirable. The values of $\bar{a}_I$ (Fig. 11b), being unequal, would indicate that an allometric equation of the type $I_{max} = cW^b$ is not truly applicable. Variation is high, however, and extra data would again be appropriate.

The effects of temperature on the stabilised filtration rates of marine invertebrates are well documented. There is general agreement that filtration rate increases with temperature until an optimum, and then declines sharply (Ali, 1970; Kinne, 1970; Schulte, 1975; Southward, 1957, 1962; Theede, 1963; Widdows, 1973a,b; Hughes, 1969). The decline occurs outside the temperature range normally experienced by the animal and should not, therefore, affect C. intestinalis in the 5-15°C range. Fiala-Médioni (1978c) found an increase in filtration rate by Phallusia mammillata, when the temperature was increased from 7-15 or 16°C. A decrease occurred at 20°C.

Fig. 12a shows that the relationship between temperature and stabilised filtration rate in C. intestinalis is not as simple as the

literature would indicate. The temperature dependence of filtration rate is related to particle concentration, such that at higher particle densities the filtration rate is independent of temperature. Temperature has the effect of altering the maximum filtration rate, as conceived in the models of filter-feeding in copepods (Lam and Frost, 1976) and lamellibranch molluscs (Winter, 1978). The maximum ingestion rate remains constant, but is not reached until progressively higher particulate concentrations with decreasing temperature. This is in contradiction to the observations of McMahon (1965) that Imax, in Daphnia magna is a function of temperature.

Q_{10}, the factor by which a reaction velocity is increased for a rise in temperature of 10°C (Prosser and Brown, 1973), obviously varies with the temperature increment examined, due to the rise and decline of filtration rate over a temperature range. It is demonstrated here that particulate concentration will also have an effect on the Q10 value. These two factors might explain the large range of Q_{10} values quoted in the literature for filtration rates. These range from 2,05 (Winter, 1969) to 6,2 (Ali, 1970) in bivalve molluscs, for a temperature change from 4°C to 14°C (or 5-15°C). McLusky (1973), however, has reported much lower values of 1,41 (5-10°C) and 1,37 (10-15°C) for Chlamys opercularis.

The range of Q_{10} values for ascidian filtration rates is even greater. Fiala-Medioni (1978c) has reported Q_{10} values of 8,34 (5-10°C), 1,57 (10-15°C) and 0,36 (15-20°C) for Phallusia mammillata, and Holmes (1973) a value of 0,54 (10-18°C) for Styela clava. The Q_{10} values of 4,00-4,34 reported here are, therefore, in the middle of the range of values found for ascidians and lamellibranch molluscs.

It is clear that body-size and temperature have well defined, but differing effects on the basic model of filter feeding of ascidians, as seen in Chapter 1. These effects are shown graphically in Fig. 5.

The axes have been changed. Body size is replaced by gut volume, this being considered the major controlling factor of maximum ingestion rate. Inorganic particulate load is replaced by seston volume, as I have found ascidians to show no preference between inorganic and organic particulates. Body-size, a metabolic determinator, has the effect, in ascidians, of translocating the basic feeding curve described in Chapter 1. Temperature, a metabolic modifier, has the effect of truncating the basic feeding curve by reducing the maximum filtration rate.

A translocation of the feeding curve, with increasing body size, has been calculated in the model of filter-feeding proposed for copepods by Lam and Frost (1976). There are, however, no reports of the effects of temperature on filtration models in the literature.

Various pollutants have inhibitory effects on the metabolism of filter-feeding poikilotherms. If a pollutant had the effect of constricting the gut, its effect on the feeding curve might resemble that of decreasing body size. This is unlikely to happen in ascidians, as the gut is essentially non-muscular. It might be expected, therefore, that pollutants having a metabolic inhibitory effect will affect the feeding curve in a similar way to decreased temperature.

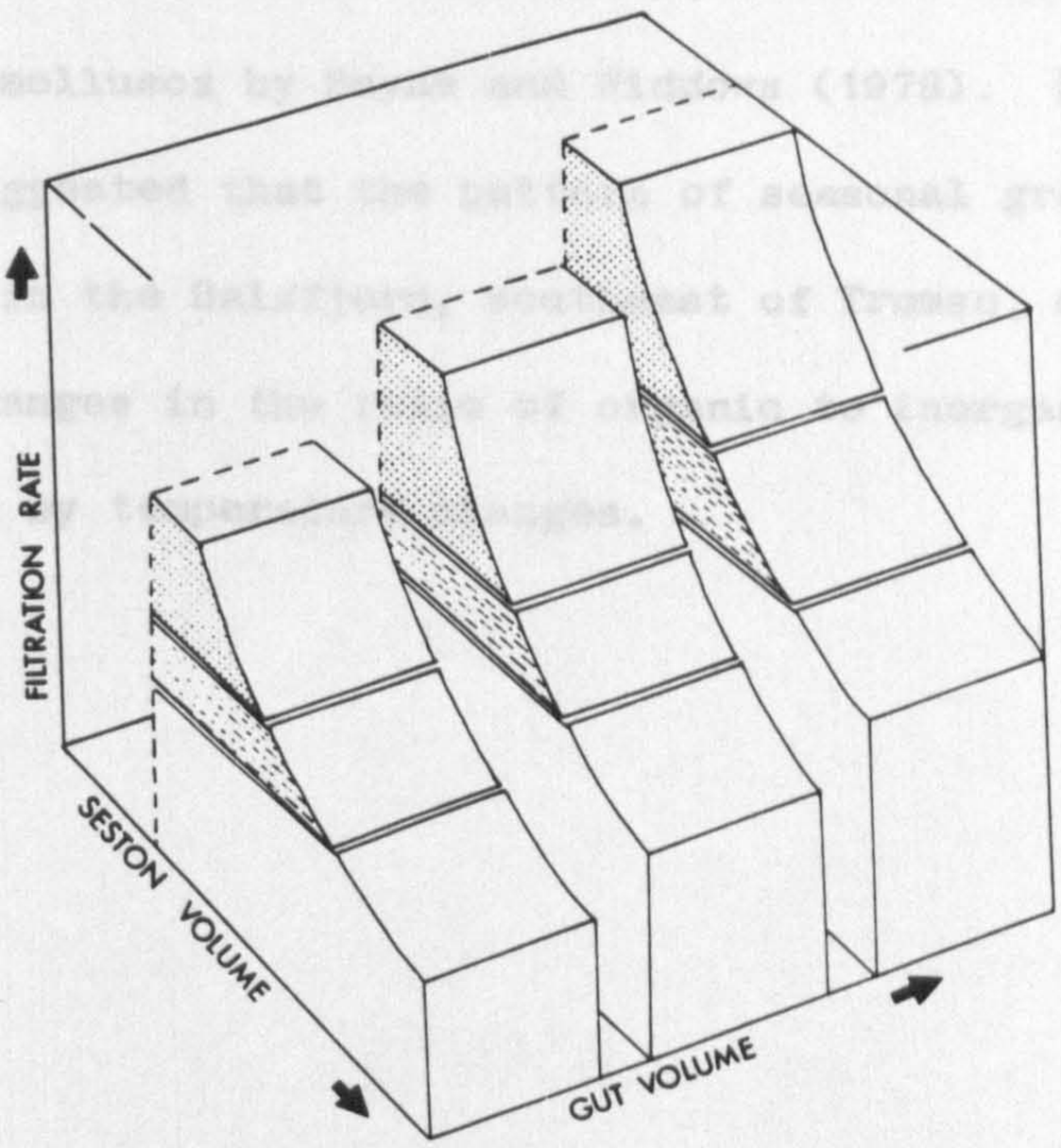
It is probable that temperature has little effect on filtration rate in the natural environment. Seston volumes in many areas probably remain high enough to satiate the animals at even the lowest temperatures, the filtration rate hence falling in the temperature independent portion of the feeding curve. Even in January, in the Clyde Sea area, ascidians are found with their guts tightly packed. Secondly, most filter-feeding poikilotherms can acclimate to temperatures within the ranges they normally experience (Widdows and Bayne, 1971; Bayne, 1973; Bayne et al., 1976; McLuskey, 1973; Widdows, 1973a,b, 1978a). Ascidians would not

FIGURE 13

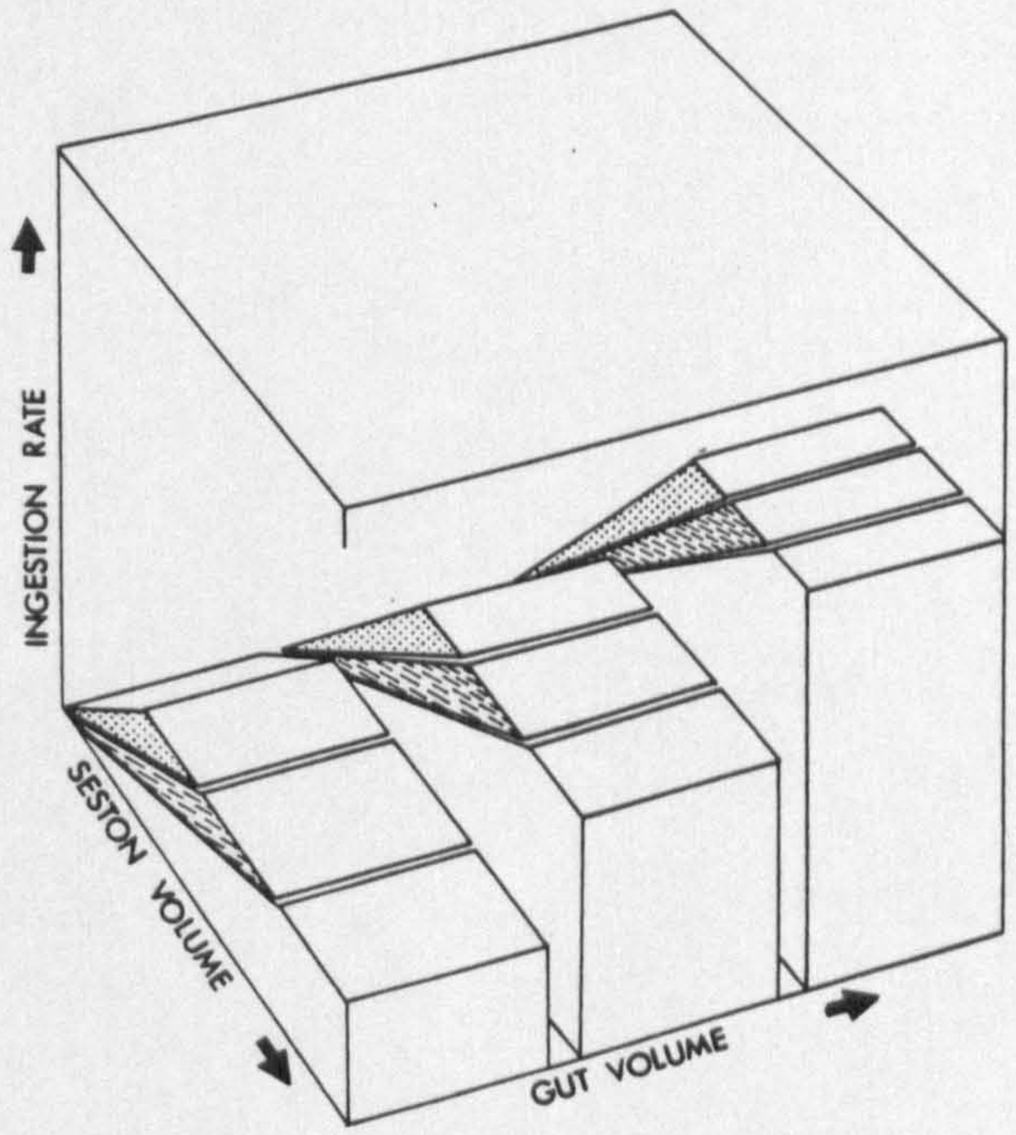
a) Model of filter-feeding in ascidians.

b) Model of ingestion in ascidians.

a.


FILTRATION RATE
SESTON VOLUME
GUT VOLUME

b.


INGESTION RATE
SESTON VOLUME
GUT VOLUME

appear to be an exception (Burkey and Farmanfarmaian, 1965). The feeding curve in the natural environment is, hence, likely to be similar at temperatures between 5 and 15°C. This phenomenon has been demonstrated for lamellibranch molluscs by Bayne and Widdows (1978). Furthermore, Dahl (1980) has suggested that the pattern of seasonal growth of Chlamys islandica in the Balsfjord, southeast of Tromso, can be explained more readily by changes in the ratio of organic to inorganic material in the seston than by temperature changes.

CHAPTER 3

"Organic and inorganic particulate suspensions and the squirting of the ascidians *Ciona intestinalis* (L.) and *Ascidiella scabra* (Müller)".

INTRODUCTION

Ascidians exhibit spontaneous activity over long periods in the laboratory (Hoyle, 1953). This activity consists of movements ranging from siphon closure to complete contraction of the mantle musculature, resulting in the discharge of about two-thirds of the volume of water from both the branchial and atrial cavities. These contractions are rapid, involving both transverse and longitudinal dimensions simultaneously. The longitudinal and circular muscles do not, though, contract antagonistically (Goodbody and Trueman, 1969). Simultaneous contraction makes the mantle wall act as a diaphragm, compressing the branchial sac, resulting in the reduction of the internal volume and a consequent jet of water expelled through one siphon. According to Goodbody and Trueman, restoration of body shape is due to the inherent elasticity of the test. Animals removed from their tests however, will recover in many cases, to feed normally (Croxall, 1971; personal observation), so restoration must be due to the inherent elasticity of the inner pharynx.

Earlier workers related this squirting response to the ejection of foreign particles, faeces or gametes (Magnus, 1902; Frohlich, 1903; Jordan, 1908), or regarded it as a resultant of external tactile stimulus. Jordan described these two responses as an 'ejektionsreflex' and a 'schutzreflex'.

The spontaneous attribute of this activity was first described by Polimanti (1910) in C. intestinalis at a temperature of 30°C, but Hecht (1918b) was the first to give a detailed account of the activity. Hecht described direct and crossed responses equivalent to the 'schutzreflex' and 'ejektionsreflex' of Jordan. These two types of response are now well documented (Yamaguchi, 1931; Hoyle, 1952).

Direct responses consist of the closing of both siphons and retraction of the body in softer tested ascidians. It is initiated by an external stimulus. The crossed response is initiated by a stimulus to the inner sides of the siphons or oral tentacles. The opposing siphon closes and the contraction of the body forces water through the stimulated siphon. Spontaneous squirting is invariably a crossed response.

The functions of spontaneous squirting are still much disputed. Hoyle (1953) calculated that squirting exchanged a greater quantity of water than pumping and, hence, suggested that its function was in feeding. Hoyle's determinations of the pumping rate, however, have been shown to be erroneous by Jørgensen (1955, 1966) and Carlisle (1966). This function is, therefore, unlikely. Jørgensen (1955, 1966) regarded squirting as a process to renew water adjacent to the exterior of the siphons and thereby prevent stagnation or any tendency to recycle water between the atrial and branchial siphons. Goodbody (1974) regarded this explanation as unlikely, since nervous control of the siphon diameter ensures that the exhalent discharge of water is driven a long way from the branchial siphon, and in most ascidians the siphons are so orientated as to ensure that mixing will not occur. Goodbody favours the idea that squirting is used to clean the branchial wall of accumulated mucus and particulate matter, and clear the atrium of faeces. The clearance of undesirable particles from the pharynx has been suggested as the function by MacGinitie (1939), Barrington (1965) and Carlisle (1966). Carlisle has likened the process to that of pseudofaecal production by filter-feeding bivalve molluscs.

Whatever the function, or functions, Moore (1977) concluded that "the subject of spontaneous squirting is one of great significance, especially with reference to the question of particle sorting and pseudofaecal production in ascidians".

MATERIAL AND METHODS

Ascidians were narcotised with crystals of menthol as outlined by Russell (1963) and Millar (1953). The ascidians were considered sufficiently relaxed when the touching of the siphon rims caused no reaction. This usually required 2-3 hours.

A fine thread was sewn into the test close to the oral siphon. The animals were attached to glass specimen jars for support (Fig. 14). C. intestinalis were pushed into small specimen tubes (s). Any tendency for the animals to be pulled out of the jars was, thus, opposed by the negative pressure created by the basal regions of the animals moving in the specimen jars. A. scabra, having a harder test was attached by a thread to a larger specimen jar (S). A small baton (b) was used to tighten the thread and secure the animal.

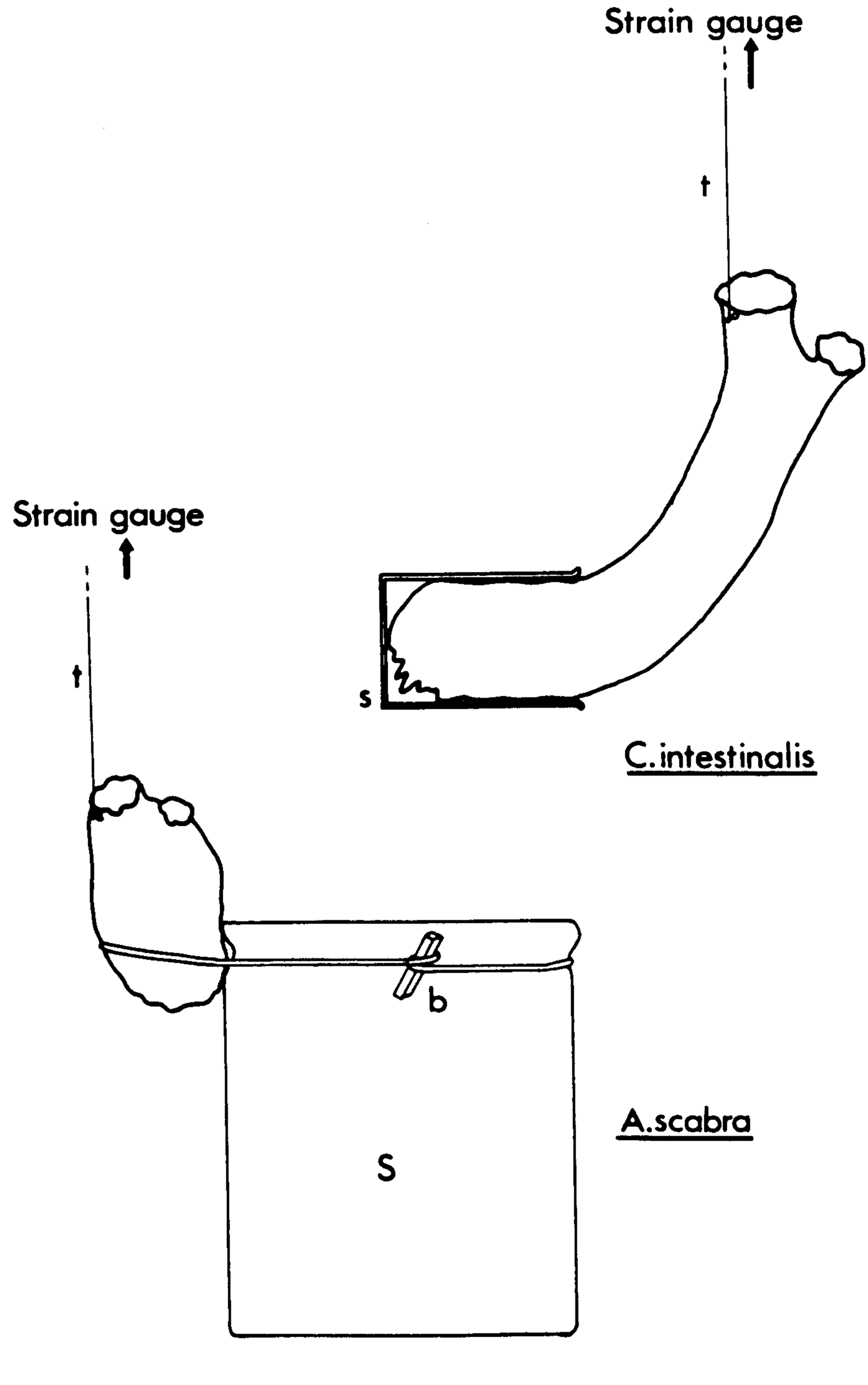
The animals were allowed to recover in running seawater for several days and finally transferred to a battery jar placed in a waterbath set at 15^{o}C. The threads from the siphon were attached to strain gauges. It was found that the best results were obtained with an isotonic strain gauge (George Washington, series 400, T1 isotonic lever transducer) for C. intestinalis and an isometric strain gauge (George Washington, series 400, D1, isometric force displacement transducer) for A. scabra. Siphonal and body movements were transduced into electrical impulses that were recorded on a George Washington series 400 flat bed recorder.

The battery jar (in the waterbath), the waterbath, the cooling unit and the retort stand holding the strain gauge were all placed on thick rubber mats to isolate the ascidians from vibrations.

The water in the battery jar was circulated from the bottom to the top with two channels of a multi-channel peristaltic pump (Schuco multi-mini pump). This kept inorganic particulates suspended without unduly disturbing the ascidians.

FIGURE 14 Attachment of ascidians for the recording of spontaneous squirting.

t = thread; s = small specimen jar;
S = large specimen jar; b = baton.


Strain gauge
t
s
C.intestinalis
Strain gauge
t
b
S
A.scabra

Animals were kept under these conditions in seawater filtered to 0,3µm (Gamma 12 in-line filter system) for 24 hours before each experiment. Experiments were of five hour duration. Experimental variables were various concentrations, and combinations of inorganic suspensions (Fuller's earth, Kaolin and Ballochmartin mud) and organic suspensions (Dunaliella salina). These suspensions were added two hours before the start of each experiment. The concentration of algal cells was estimated with a haemocytometer (Neubauer B.S. 748). Inorganic suspension load was estimated with an absorbtiometer (see Chapter 1). Concentrations were estimated at time 0 and time 5 hours, an average of the two being taken as the mean concentration.

The mean squirting rates, for each set of experimental conditions, were calculated as the average of the results over 5 hours for 7-8 animals.

24-hour experiments

Two sets of 24 hour experiments were conducted with C. intestinalis. One was conducted in mid-summer at 15°C, the other in mid-winter, also at 15°C. The animals were allowed to acclimate to the temperature of 15°C for several days in each case.

RESULTS

a) The squirting reaction

Examples of recordings are given in Fig. 15. These illustrate some of the variations found in the basic squirting reaction. This basic response is best illustrated by *A. scabra* (mud) (Fig. 15c). This involved a sharp contraction, reaching a plateau, and a somewhat slower recovery. The sharp contraction was also found with *C. intestinalis* (Fig. 15b), the plateau region was, however, negligible and the recovery had an initial fast rate followed by a second, slower, recovery period. These differences are, presumably, due to the fact that squirting in *C. intestinalis* involves movement of the whole body, whereas in *A. scabra* it involves only the siphons and internal components of the pharynx.

The recovery period was sometimes greatly prolonged (Fig. 15d).

The amplitude and duration varied, but often larger responses were interspersed with smaller ones (Fig. 15a). When inorganic particulate suspensions were present in the water, a series of step-wise contractions were sometimes observed, often culminating in closure of the siphons for a period (Fig. 15e). This response occurred both with *C. intestinalis* and *A. scabra*.

b) 24-hour experiments

The rate of squirting in *C. intestinalis* oscillates about a mean (Fig. 16). In summer there was a marked increase in the rate of squirting at dawn and, to a lesser extent, at dusk. During these periods of increased activity, which lasted about thirty minutes, there was a release of gametes visible as white clouds (Fig. 16a). In the experiments conducted in winter there was no increase in the rate of squirting at dawn and dusk, and no gamete release.

FIGURE 15 Examples of squirt traces for ascidians.

a. A. scabra (mud)

b. C. intestinalis

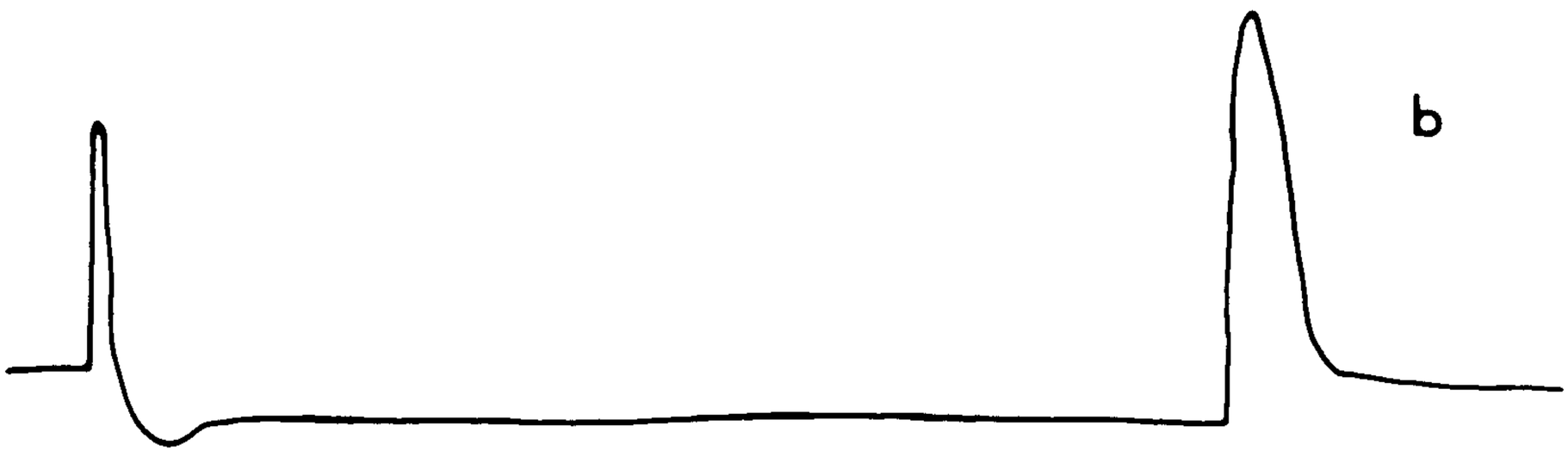
c. A. scabra (mud)

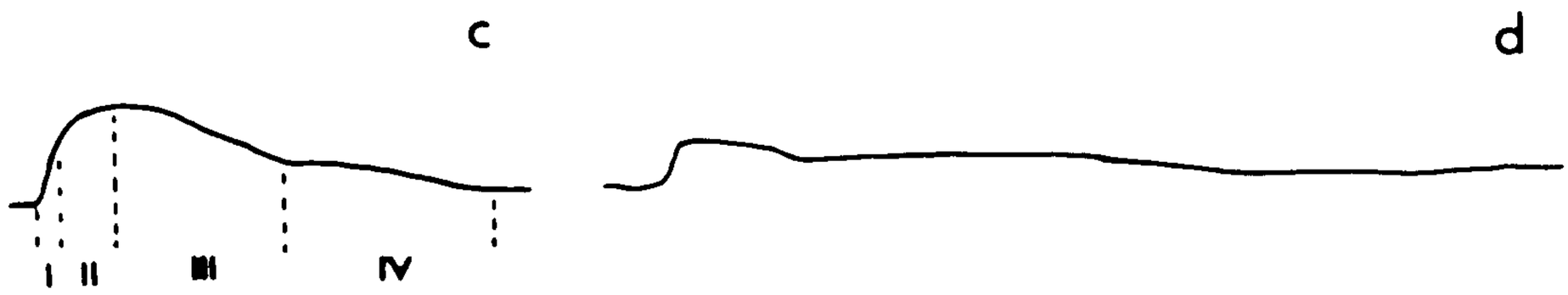
d. A. scabra (mud)

e. A. scabra (mud)

In c. the roman numerals represent the four phases of body movement described by Hecht (1918a).


a


b


c
I
II
III
IV
d

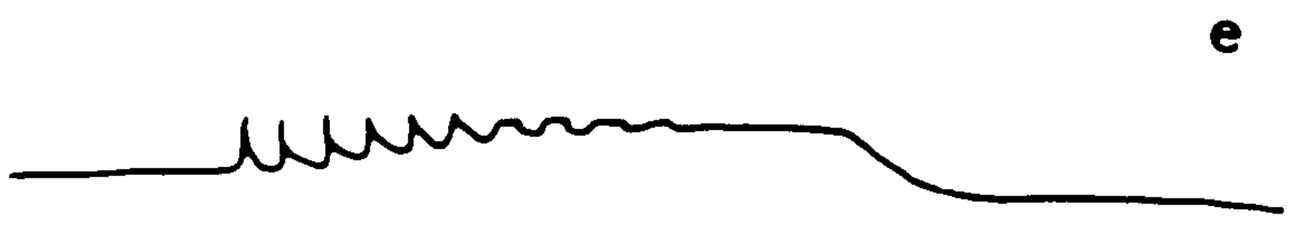
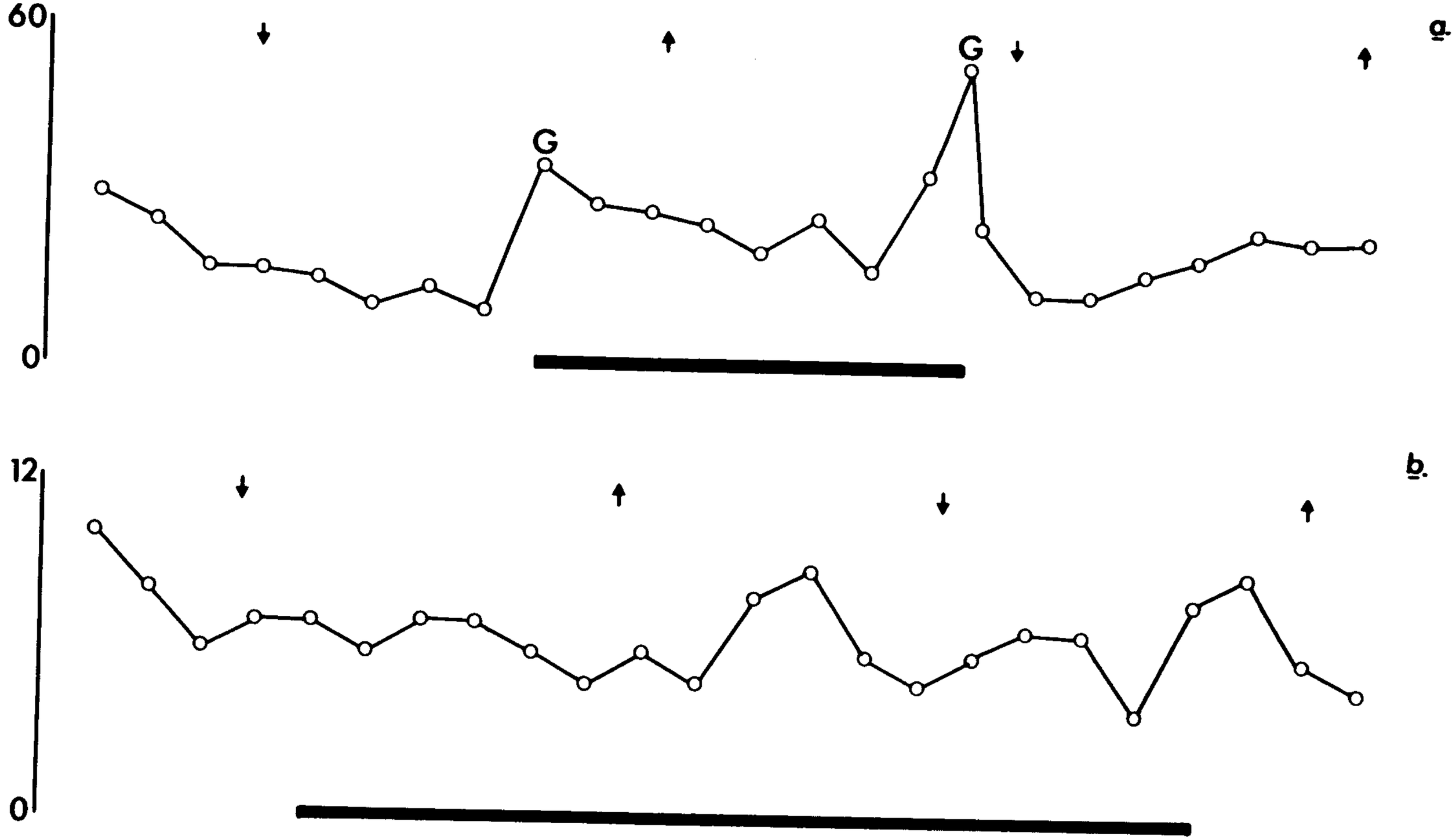

e

FIGURE 16 Squirting rates of C. intestinalis at 15°C over 24 hour periods.

a. summer

b. winter

Black bars represent the periods of darkness and arrows the times of low and high tides.
G = gamete release.


60
0
G
G
a.
12
0
b.

PHASE	PRESENT STUDY	RESULTS OF HECHT (1918a)
I	5,0	7,0
II	10,4	17,8
III	33,0	22,8
IV	41,0	123,0

TABLE 12 Duration (seconds) of the four phases of the body contraction response as described by Hecht (1918a).

AUTHOR	SPECIES	TEMPERATURE	SQUIRTS.h^{-1}
Hecht (1918a)	*Ascidia atra*	?	17-20
Yamaguchi (1931)	*Styela clava*	?	8-27
Hoyle (1953)	*Phallusia mammillata*	11-14°C	7-10
Buisson et al (1976)	*Ciona intestinalis*	17-19°C	20
Shumway (1978)	*Ciona intestinalis*	10°C	14

TABLE 13 Rates of spontaneous squirting found for ascidians in previous investigations.

The normal squirting rate in the summer experiments was elevated due to several animals touching each other, the movement of one causing a direct response in the others. In the winter experiments, the animals were held individually.

c) The effects of particulate suspensions

The mean rates of squirting in filtered seawater were 9 squirts h^{-1} for C. intestinalis, 8 squirts h^{-1} for A. scabra (Fucus) and 12 squirts h^{-1} for A. scabra (mud), (Figs. 17-19).

Increasing algal cell concentration caused slight increases in the rate of squirting of C. intestinalis and A. scabra. Greater increases were initiated by increasing suspension loads of inorganic particulates. The higher concentrations of inorganic particulates more than doubled the squirting rate found in filtered seawater for C. intestinalis and A. scabra (Fucus). The rate of A. scabra (mud) was slightly less than doubled. Comparable results were obtained with Fuller's earth, Kaolin and Ballochmartin mud. The squirting rates remained elevated throughout most of the 5 hour experimental period, when particulate suspensions were present in the water. There was a tendency for a slight decrease in rate, especially towards the end of the 5 hours (Fig. 20).

The average duration of squirts remained more or less constant in C. intestinalis (Fig. 21a). That of A. scabra (mud) was far more variable. This variability, however, is not related to particle density (Fig. 21b). The average duration of squirts of A. scabra (Fucus) remained constant over most of the range of particulate suspension concentrations tested, but increased at the highest concentrations (Fig. 21c).

The product of average squirt duration and the rate of squirting is considered as the time loss caused by squirting. This loss is expressed as a percentage of the experimental period (Fig. 22). An increase in the percentage time loss was found with increasing particulate

concentrations for both C. intestinalis and A. scabra, this increase being least pronounced for A. scabra (mud).

FIGURE 17 Squirting rates S(squirts h^{-1}), of C. intestinalis exposed to various concentrations of algal cells, A(cells l^{-1}) and inorganic particulates ($mg.l^{-1}$).

FE = Fuller's earth
K = Kaolin
BM = Ballochmartin mud.

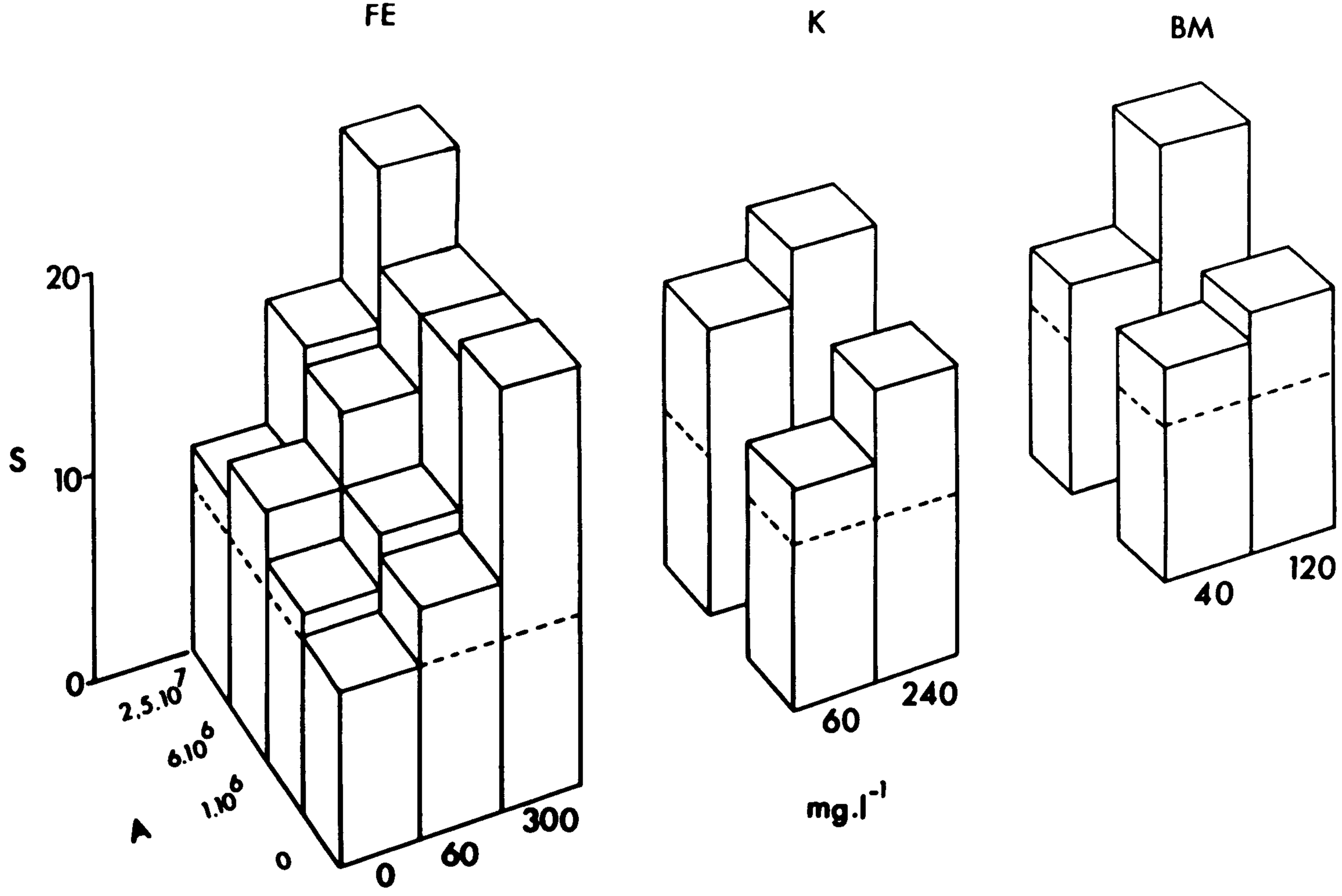

FE
K
BM
20
S
10
0
2.5.10^7
6.10^6
1.10^6
A
0
0
60
300
60
240
40
120
mg.l^-1

FIGURE 18 Squirting rates, S(squirts h^{-1}), of A. scabra (mud) exposed to various concentrations of algal cells, A(cells l^{-1}) and inorganic particulates ($mg.l^{-1}$).

FE = Fuller's earth
K = Kaolin
BM = Ballochmartin mud.

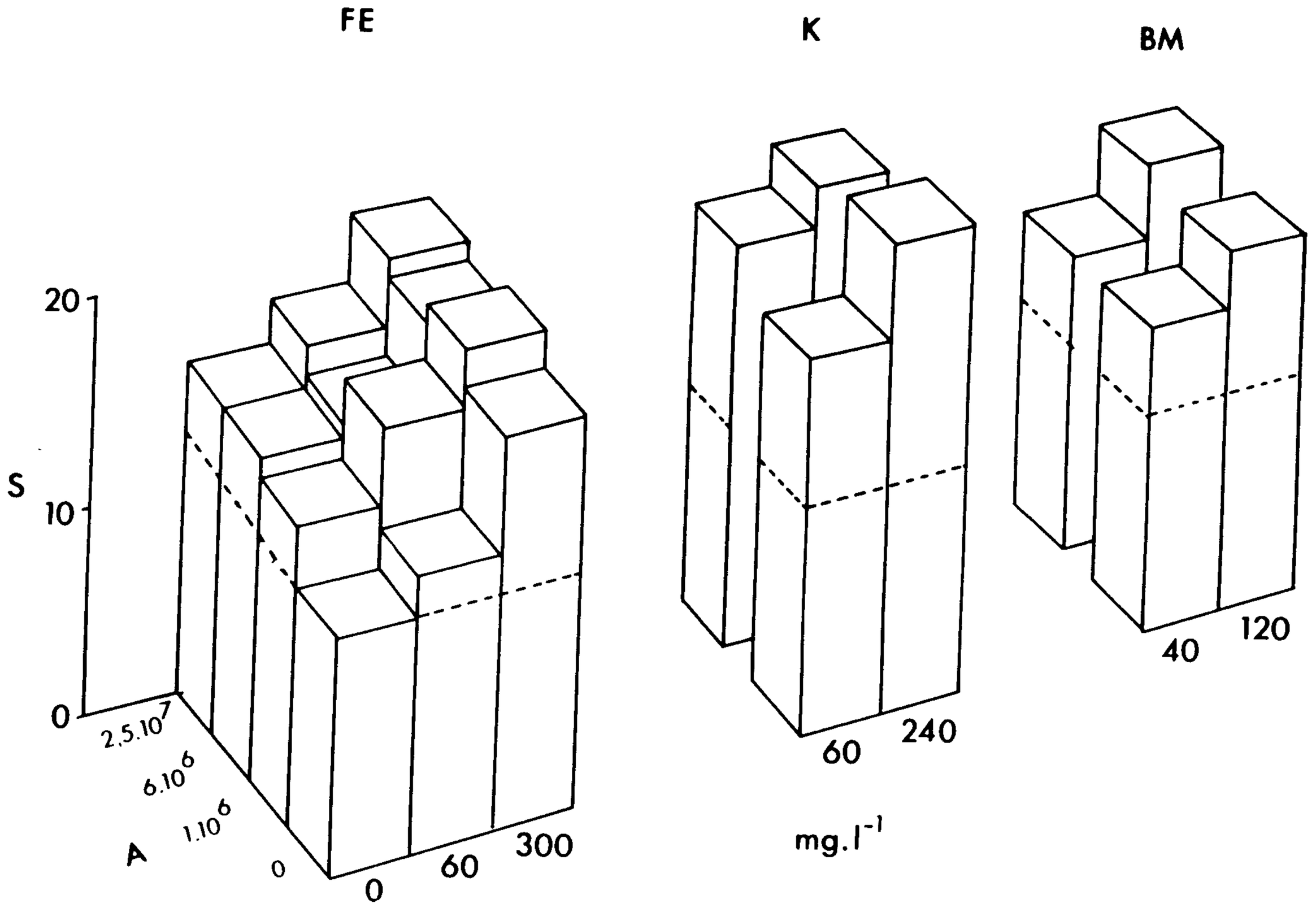

FE
K
BM
20
S
10
0
2.5.10^7
6.10^6
1.10^6
A
0
0
60
300
60
240
40
120
mg.l^-1

FIGURE 19 Squirting rates, S(squirts h^{-1}), of A. scabra (Fucus) exposed to various concentrations of algal cells, A(cells l^{-1}) and inorganic particulates.

FE = Fuller's earth
K = Kaolin
BM = Ballochmartin mud.

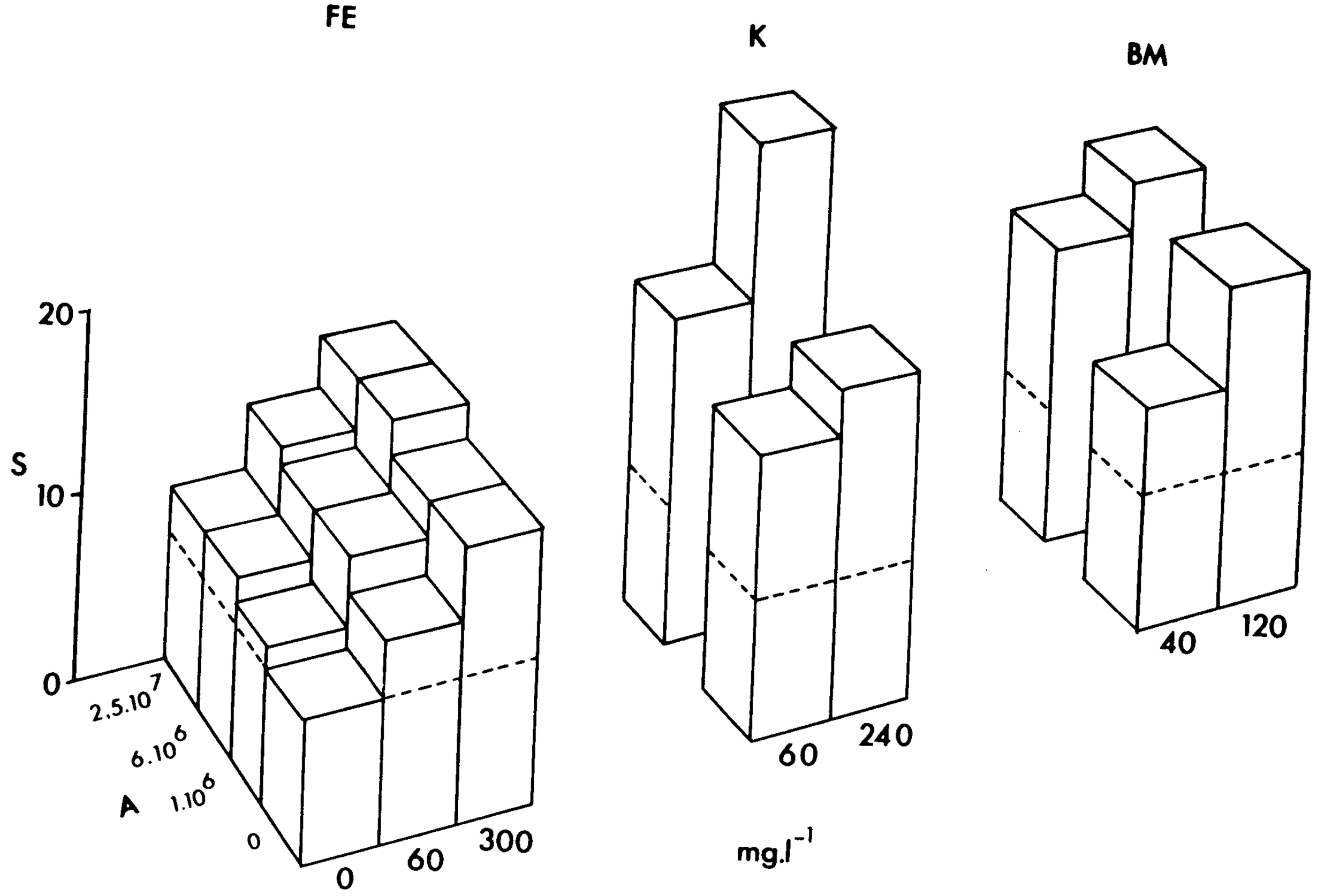

FE
K
BM
20
S
10
0
2.5.10^7
6.10^6
A
1.10^6
0
0
60
300
60
240
mg.l^-1
40
120

FIGURE 20 Squirting rates (squirts h^{-1}) of ascidians exposed to suspensions of Fuller's earth:

○ = 300 $mg.l^{-1}$

● = 60 $mg.l^{-1}$,

and algal cells ($cells.l^{-1}$).

Horizontal lines are average rates in filtered seawater (with standard errors - broken lines).

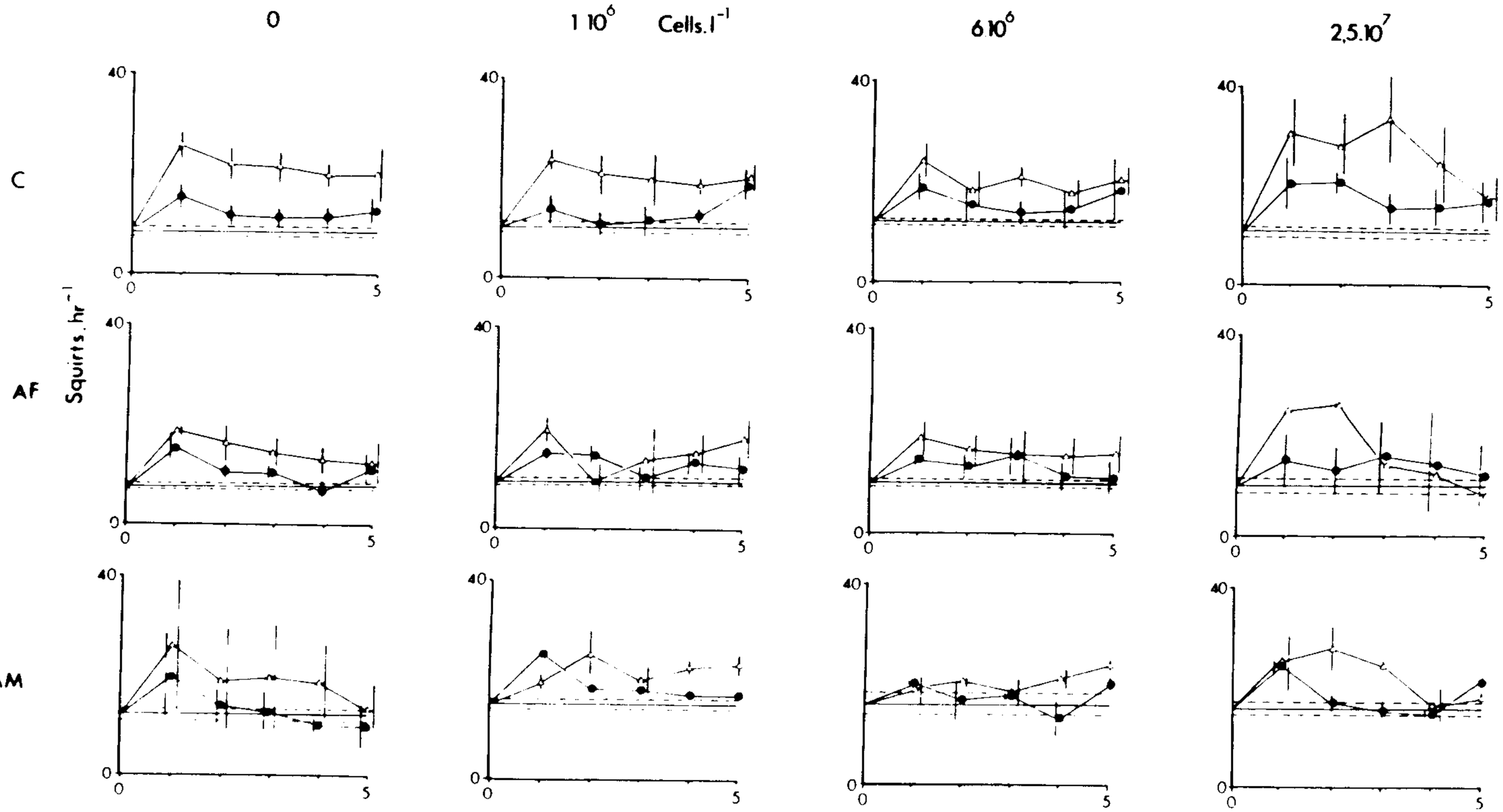
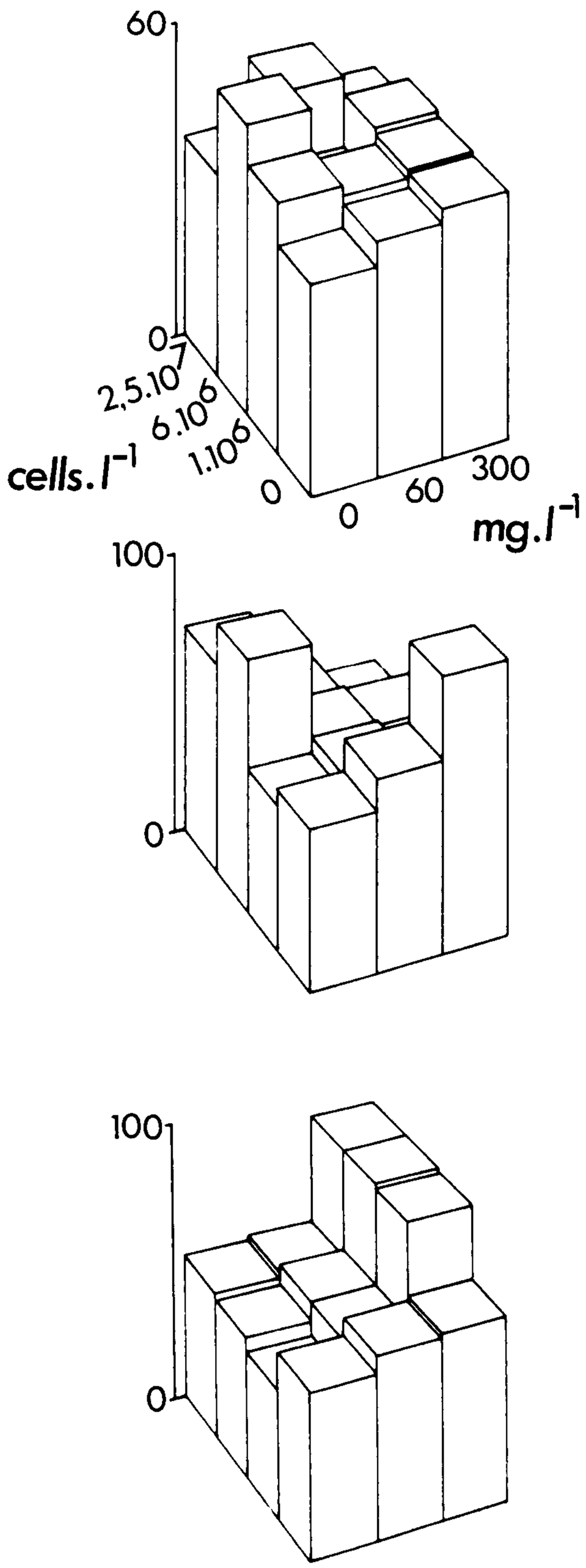

0
1 10^6 Cells.l^-1
6.10^6
2,5.10^7
C
AF
AM
Squirts.hr^-1
40
0
5
Hours

FIGURE 21 Average duration (seconds) of squirts of ascidians exposed to suspensions of Fuller's earth ($mg.l^{-1}$) and algal cells ($cells.l^{-1}$).

a. *C. intestinalis*

b. *A. scabra* (mud)

c. *A. scabra* (*Fucus*)



a

b

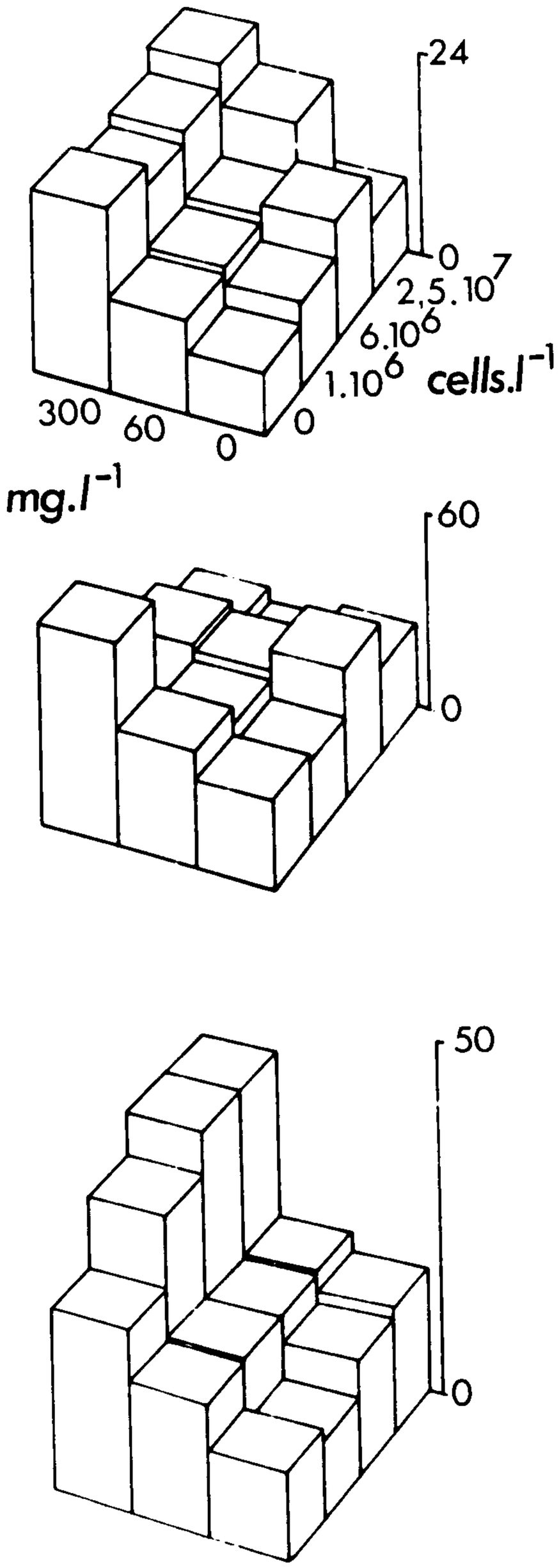
c

FIGURE 22 Mean percentage time loss due to squirting of ascidians exposed to suspensions of Fuller's earth ($mg.l^{-1}$) and algal cells ($cells\ l^{-1}$).

a. *C. intestinalis*

b. *A. scabra* (mud)

c. *A. scabra* (*Fucus*)



a

b

c

DISCUSSION

The basic squirting response (Fig. 15c) is identical to that described by Hecht (1918a). The four phases of the response, as described by Hecht, are illustrated. The duration of the phases in Fig. 15c are compared with those found by Hecht for *Ascidia atra* in Table 12. The results are similar, although Hecht found a longer recovery period. The recovery period in the present study, however, was variable and sometimes greatly increased (Fig. 15d). Hoyle (1952) found a similar prolonged relaxation period, in fatigued animals, and Polimanti (1910) reported that the rate of relaxation is affected by uncontrollable factors. Variations in the degree of response (Fig. 15a) have been reported by Hoyle (1952) and large contractions interspersed with several smaller ones by Hecht (1918a), Yamaguchi (1931) and Hoyle (1953). Hecht explained this finding as caused by complete body movements interspersed by siphon rim movements (complete body movements are, presumably equivalent to complete pharyngeal movements in hard tested ascidians such as *A. scabra*). The present results would indicate that there is a range in the degree of responses, and not simply large and small responses. Hoyle (1952) found a similar phenomenon using physical and electrical stimuli and concluded that the pattern of spontaneous activity affects the properties of the nerve-muscle system, such that similar stimuli may elicit different degrees of response in the same animal at different times. Furthermore, stimuli given immediately after a spontaneous squirt frequently give rise to one, or a series of, smaller contractions, of variable amplitude, at short intervals (Kinoshita, 1910; Hoyle, 1952). In these experiments the stimuli were presumably produced by the particulate suspensions in the water.

I can find no reference, in the literature, to the step-wise contractions, illustrated in Fig. 15e, involved in spontaneous squirting under normal conditions. Hoyle (1952), however, reported a phenomenon of facilitation in Phallusia mammillata with physical and electrical stimuli. This process involved a second stimulus producing a larger reaction due to an extensive facilitation consequent upon the arrival of a first effective stimulus. When several stimuli were received at short intervals, a typical smooth-muscle type staircase response was produced. Facilitation is easily exhausted (Hoyle, 1952), which might explain the "staircase" response in this study culminating in a short period of siphon closure.

Buisson and Fournier (1976) reported an increase in the rate of squirting of C. intestinalis during a 15-30 minute period at dawn. During this period the squirts comprised the closing of the oral siphon and the contraction of the thorax causing the expulsion of a cloud of white material from the exhalent siphon. They did not investigate the nature of this material, but speculated that it is the cellular material exuded by the neural gland as part of its rhythmical change from a compact to a reticulated phase (Georges, 1971). Georges was uncertain of its fate. Buisson and Fournier suggested that this phenomenon represents a rhythmical behaviour, but were not certain whether this rhythm is endogenous or exogenous.

Increases in activity in the summer were found both at dawn and, to a lesser extent, at dusk in the present study. In both cases, the duration was similar to that found by Buisson and Fournier. Similarly the closing of the oral siphon and expulsion of a cloud of white material from the exhalent siphon was observed. The white cloud, however, was identified as gametes. No similar increases were found in the activity of C. intestinalis acclimated to 15^{o}C in winter. The animals in winter

do have ripe gonads (Millar, 1953) but, even after the period of acclimation to 15°C, do not appear to release gametes. These activity increases would, therefore, appear to constitute a gamete expulsion process; the correlation with changes in light intensity facilitating the simultaneous release of gametes by large numbers of animals. This mechanism is enhanced by the phenomenon discovered by Carlisle (1951), whereby gametes released by one animal, detected by the neural gland of another, induce the release of gametes by the latter. The release of gametes at dawn has previously been reported by Berrill (1947) and correlated to increases in light intensity by Lambert and Brandt (1967) and Whittingham (1967).

The average rates of squirting found for C. intestinalis and A. scabra in filtered seawater are lower than rates found in seawater by other authors, with the exception of the rates found by Hoyle (1953) in Phallusia mammillata (Table 13). But Hoyle (1953) and Yamaguchi (1931) have both found an increase in rate with filtered seawater, which they have proposed as a starvation response. This response was not found in these experiments, perhaps because the animals were allowed to acclimate to the filtered seawater for 24 hours before experimentation. This starvation response led Hoyle to suggest that the rate of spontaneous squirting was inversely proportional to food concentration. The complete opposite was, however, found in this study.

Increases in squirting rate are consistent but slight when algal cells were present in the water. Far greater increases were found with inorganic particulates. Expressed as particle packing volumes (Table 2 in the General Introduction), 60 $mg.l^{-1}$ Fuller's earth is greater than $2,5x10^{6}$ cells l^{-1} Dunaliella salina. When viewed in this light, there is a progressive increase in the rate of squirting with the volume of particles in the water column. Squirting rate, hence, is directly proportional to the volume of particles in the water. Reports of

initiation of squirting, by particulates in the water, have in earlier investigations been limited mainly to excessively large particles (MacGinitie, 1939; Werner and Werner, 1954), or 'particles of a nature foreign to the usual run of food material' (MacGinitie, 1939). Goodbody and Trueman (1969) found no effect of graphite particles on the squirting rates of Ascidia nigra and Ascidia interrupta. Unfortunately, they give no indication of the graphite concentration, which I suspect to be rather low. Hoyle (1953) reported that in very thick suspensions of carmine, some of the particles were ejected from the branchial siphon by squirting. Jørgensen and Goldberg (1949) found that excessive (i.e. several $mg.l^{-1}$) amounts of graphite caused violent squirting with the graphite-laden, mucous feeding sheets being blown out of the branchial siphon. This extreme reaction was observed but rarely in the present study, and only at the highest concentrations of the inorganic particulates.

The volumetric concentrations of the particulates used in this study are approaching, or above, those at which ascidians can satiate their guts (see Chapter 1). Due to the small increases in squirting rate at the lower range of volumetric concentrations used, it seems reasonable that increased squirting rate is mainly associated with excessive particle concentrations. The fact that an increase is found with increasing concentrations of unicellular algae would indicate that the response is not to particle quality, but to particle quantity.

The smaller increases in the squirting rate of A. scabra (mud) in response to excessive inorganic particulate suspensions are due, not to reduced increases at high particulate levels, but to elevated rates in filtered seawater. A. scabra from this environment might be expected to encounter more large particulates than animals attached to Fucus fronds.

It is possible that this might entrain a higher rate of spontaneous squirting.

The slight decreases found in the squirting rates over the 5hr experimental periods show no abruptness and are thought to be due to decreasing particulate concentration.

There was a great variation in the duration of squirts, akin to that of 9-76, 25 seconds (according to the number and intensity of stimulations given) found by Kinoshita (1910). The average duration remained essentially constant for C. intestinalis and A. scabra (mud), an increase being found only with A. scabra (Fucus) at higher particulate loads. This apparent difference might be explained by the smaller size of the A. scabra (Fucus), smaller animals becoming satiated at lower particulate concentrations than larger ones (Lam and Frost, 1976).

The overall effect of squirting is to reduce the percentage of time available for filtration. The percentage time loss increases, in C. intestinalis at concentrations of algal cells of $1x10^6$ cells l^{-1} and above. In A. scabra it increases at concentrations of $6x10^6$ cells l^{-1} and above. The increased squirting rates may, hence, explain, at least in part, the reductions in filtration rates reported in the first two chapters.

The function of squirting has been a matter of dispute. It would seem likely that several functions are served by the squirting reaction. The spontaneous squirting observed in filtered seawater, in this study, is considered to have a different function, or functions, to that of the increased rate observed at higher particulate concentrations. The increased rate would appear to function in reducing the intake of particulates once the gut has become satiated, and in this sense is in agreement with the observation that squirting can be elicited by overloading of the filters (Jørgensen 1954, 1966). Squirting, however,

can also be initiated by chemical stimuli or larger particles impinging on the oral tentacles (Day, 1919; Werner and Werner, 1954; Croxall, 1971; personal observations).

Spontaneous squirting in filtered seawater, and low concentrations of particulate matter, cannot be explained by either of the above functions. Defaecation is achieved by squirting (pers. obs.) and it would seem likely that this is one function of spontaneous squirting, as has already been suggested (Jordan, 1908; Day, 1919; and MacGinitie, 1939). Jordan observed that during spontaneous squirting it is frequently the oral siphon that closes and the cloacal that remains open. Goodbody (1974) also regarded spontaneous squirting as clearing faeces and particulate material from the atrial cavity, thus enhancing the sanitary processes of the cavity. Garstang (1891) described pharyngo-cloacal slits in large specimens of Ascidia mentula and has suggested that this complements the water expulsion from the branchial to atrial cavity to clear faeces.

Spontaneous squirting has been associated with particle sorting by MacGinitie (1939) who has suggested that large particles trapped by the mucus can, in some way, be dropped from it and expelled by squirting. Millar (1960) has given further evidence of this phenomenon with his discovery that the pharynges of Distaplia cylindrica and Eugyra aernbaekae contained a mixture of sand and cells of phytoplankton, whereas the stomach contained only the cells. Monniot and Monniot (1978) have pointed out, however, that animals caught with a dredge may be filled with sediment at the moment of capture. This sorting process has been likened to the pseudofaeces production of filter feeding bivalve molluscs by Carlisle (1966). I can find no evidence of particle sorting in C. intestinalis and A. scabra, the contents of the guts mirroring the suspended material in the water. Squirting would not seem

to be analogous to pseudofaecal production on this count. The true function of pseudofaecal production in bivalves, however, has recently been suggested to be a bypass for food once the gut is full and not, as previously thought, a disposal mechanism for unwanted particles sorted from the feeding currents on the gills (see review by Winter, 1978). Some particle selection may occur during pseudofaecal production (Kiørboe et al., 1980; Kiørboe and Møhlenberg, 1981) but only once the gut is satiated. In this context, the increased rate of squirting in ascidians has a similar function to pseudofaecal production in bivalve molluscs. There is, however, a great difference in action. Squirting disrupts the normal feeding currents, whereas pseudofaecal production does not. In this, respect, squirting bears some similarity to the increased shell movements of bivalve molluscs exposed to increased concentrations of particulate matter (Loosanoff, 1962; Loosanoff and Tommers, 1948).

CHAPTER 4

"The effects of inorganic particulate suspensions on the rate and periods of pumping of the ascidian Ascidia mentula Müller".

INTRODUCTION

The filtration rates of simple ascidians decrease in response to increased inorganic particulate suspensions (Chapters 1 and 2), whilst the time lost due to squirting increases (Chapter 3). It is logical to assume that the increased time loss accounts for the decrease in filtration rate. When the data are compared (Table 14), however, it becomes apparent that the increased time loss accounts only in part for the reduced filtration rate. In order to explain this discrepancy, pumping rates of the ascidian A. mentula were estimated by a direct method. A. mentula was chosen as it is large, rounded and has a smooth, relatively hard test. The direct method involved the use of a split-chamber constant-level apparatus.

The constant level apparatus was devised by Galtsoff (1926) and has since been used in various forms by several authors (reviewed by Hildreth (1976) and Ali (1970)). Various means of automatically monitoring the rate of flow of water from the apparatus have been used by previous authors. Loosanoff and Engle (1947) and Hildreth (1976) used counter balanced dumping vessels. Arudpragasam and Naylor (1964) made use of a periodic siphon with a float mechanism. This apparatus was modified by Taylor (1976) with the use of a transistorised relay activated pump. None of the above methods measure the absolute pumping rate (i.e. the rate when pumping), but instead measure the nett rate (i.e. the rate over a given time period, which may include periodic stoppages). The method of Davids (1964) involving two pin electrodes that register the number of drops passing over them can be used to measure the absolute pumping rate, but was found unsuitable in these experiments due to the high pumping rates involved. A periodic siphon, similar to that of Arudpragasam and Naylor, was hence used. The

	C. intestinalis	A. scabra (mud)	A. scabra (Fucus)
% reduction in filtration rate	65,4	64,1	75,0
% increase in time loss due to squirting	50,9	43,7	28,5

TABLE 14 Comparison of the percentage reduction in filtration rate, and percentage increase in time loss due to squirting, between 60 and 300 $mg.l^{-1}$ Fuller's earth (calculated from Chapters 1 and 3).

mechanical float mechanism, however, was replaced by carbon electrodes.

n addition a flow detector was added such that absolute pumping rates

ight be calculated.

MATERIAL AND METHODS

i. Apparatus

The apparatus used is illustrated in Fig. 23. The constant level apparatus consisted of two plastic chambers joined by a large bore (5cm) plastic tube. Each chamber had a stand pipe for overflowing water (O_1 and O_2). The stand pipes were positioned such that they reached the same height above the base of the apparatus, when the chambers were level. The constant level apparatus was mounted on a base board, the level of which could be adjusted with screw a. Water was pumped from a large reservoir (R) by a pump (P) into one of the chambers of the constant level apparatus. Water in the reservoir was agitated by a large vertically reciprocating perforated plate (S) as described by Schubel et al., 1972. The plate was driven by a motorised cam mechanism as described by Shillaker (1977). This mechanism is shown in Fig. 24b. The plate moved up and down at a rate of 18 cycles per minute.

A rubber sleeve (s) was constructed from a balloon. The larger opening of the sleeve was sealed around the plastic tube separating the chambers. Large specimens of A. mentula were chosen and cleared of epibiotic growth. An animal was pushed through the rubber sleeve, such that the sleeve sealed around its test. The inhalent and exhalent siphons were thus separated between the two chambers of the constant level apparatus, the inhalent siphon being in the chamber receiving the water input from the reservoir.

Excess water pumped into the inhalent chamber of the apparatus overflowed (via O_2) back into the reservoir. Any water pumped by the animal overflowed via O_1 into a flow meter and finally back to the reservoir.

FIGURE 23 General layout of apparatus

CR = chart recorder

F = flow meter

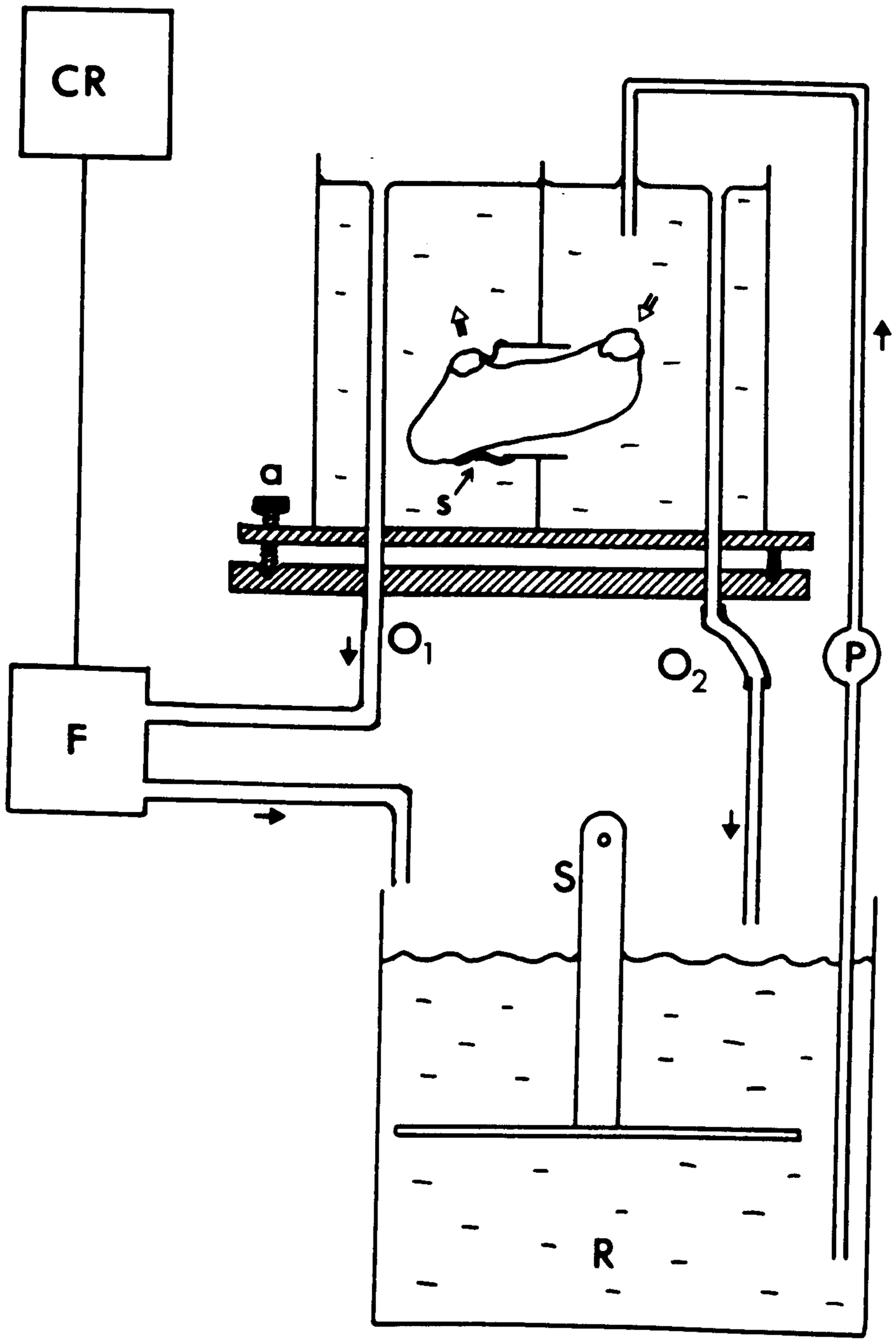
O_1 and O_2 = overflows

P = pump

R = reservoir

s = rubber sleeve

S = stirrer

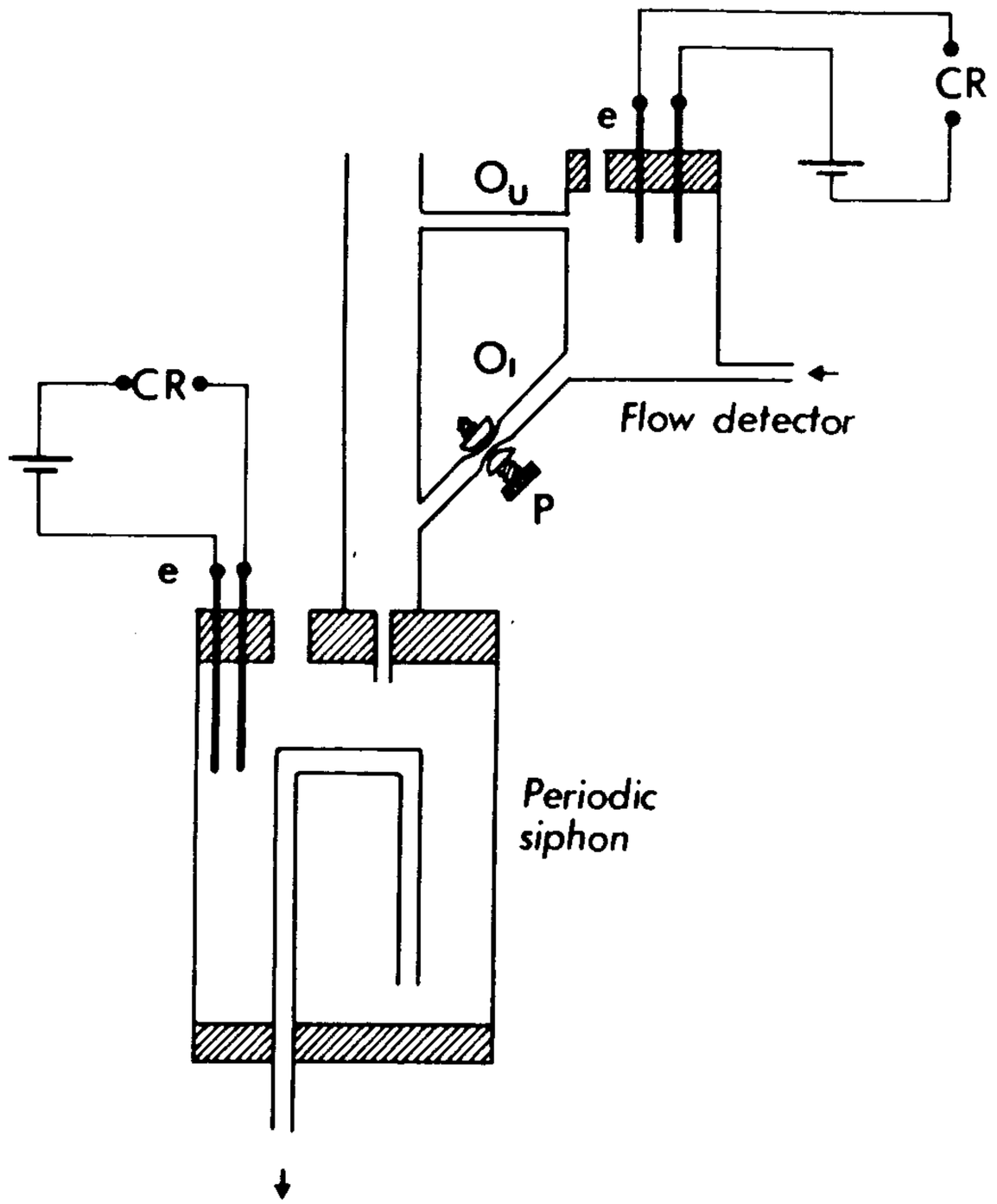

CR
F
a
s
O1
O2
P
S
R

The flow meter is illustrated in Fig. 24a and consisted of two parts. The first part was a flow detector. This was constructed from a small specimen tube with two outlets, O_l and O_u. O_l was of a large enough bore that all water entering the tube could overflow through it. The overflow from O_l could, however, be reduced with an adjustable pinchcock, P. P was adjusted such that when the animal was pumping, O_l was not sufficient for all of the water entering the tube to overflow from it. The water level in the tube, hence, rose and excess water overflowed via O_u. Under such conditions contact was made, by the water, between two carbon electrodes (e_1), completing an electrical circuit and registering on a chart recorder (George Washington, series 400, flat bed recorder). When the animal stopped pumping, the water level in the tube dropped as the water remaining in the tube could overflow via O_l. Contact between the two carbon electrodes was hence broken. Water leaving the flow detector via O_l and O_u was collected and fed into the second part of the apparatus, a periodic siphon. When the water level in the siphon reached the top of the inverted U-tube, it siphoned out. Shortly before the siphoning commenced, the water made contact between two carbon electrodes (e_2) completing a second electrical circuit and registering on a chart recorder. During the siphoning period, water is constantly entering the periodic siphon chamber. The quantity of water expelled during each siphon is, hence, not equivalent to the volume of the siphon, but changes according to the input rate into the siphon chamber. For this reason the periodic siphon was calibrated prior to experimentation (Fig. 25).

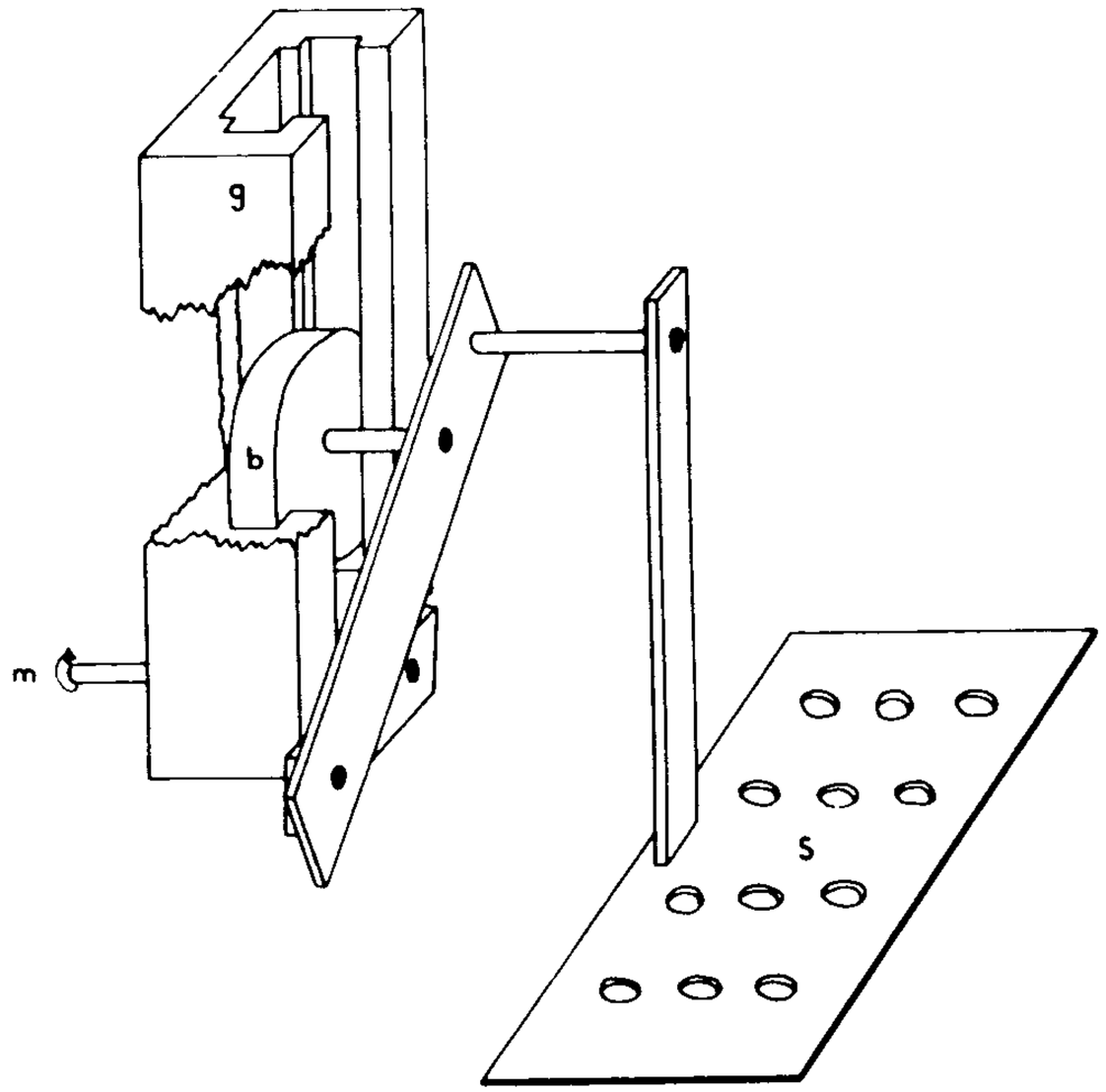
ii. <u>Preliminary adjustments of the constant level apparatus</u>

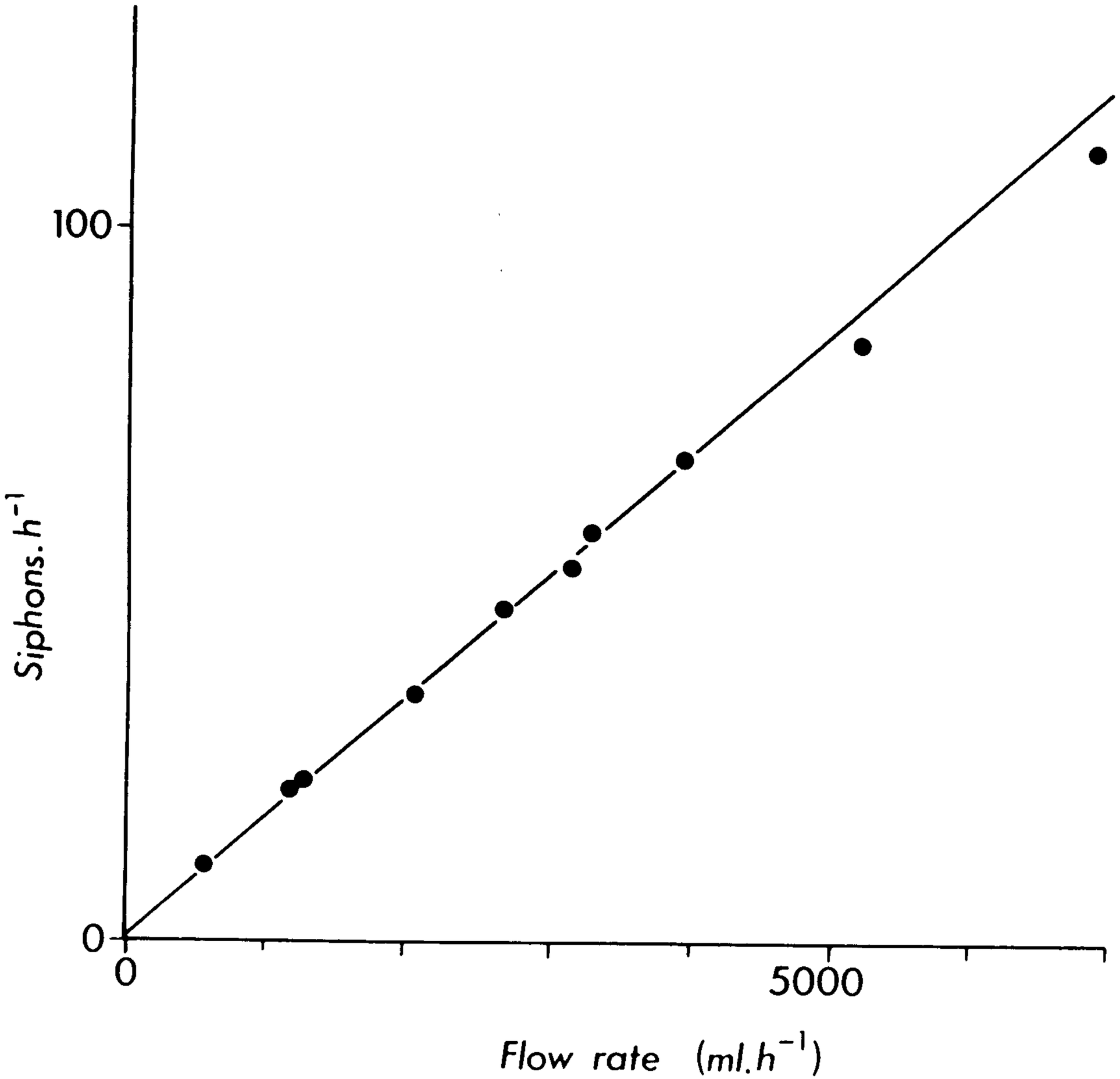
Two schemes of preliminary adjustment have been followed by previous authors, the merits of which are discussed by Hildreth (1976). The first scheme, illustrated in Fig. 26a, is that of Davids (1964). With

a.



b.




100
0
0
5000
Siphons. h^{-1}
Flow rate ($ml.h^{-1}$)

the connecting tube between the two chambers open (i.e. with no animal), the level of the apparatus is adjusted such that the overflows O_1 and O_2 are equal. There is thus a dynamic flux of water between the two chambers (F). When the animal is in position and pumping, there is therefore, a hydrodynamic force acting to exaggerate the pumping rate. The second scheme (Fig. 26b) is that of Galtsoff (1926). With the connecting tube open, the apparatus is tilted such that water just ceases to overflow via O_2. F therefore becomes 0. For the animal to pump water it must increase the water level in the exhalent chamber for it to overflow from O_2. There is, therefore, a difference in the water levels of the two chambers (Δh), causing a hydrostatic force acting to diminish the pumping rate.

Hildreth and Mallet (1980) overcame these problems by having water inputs to both chambers. In the present study, however, this was undesirable, as a visible comparison of the turbidity of the two chambers was required.

The problem with Galtsoff's method lies with the fact that the water level in the exhalent chamber must be raised to overcome the surface tension effect around the stand pipe rim. Once this effect is overcome and water starts to overflow, further increases in the rate of water passage between the two chambers will produce a negligible increase in the water level of the exhalent chamber (provided that the overflow rate is not so great that an airspace is not maintained within the stand pipe, which did not occur in these experiments). Adjustments were, hence, made in these experiments as follows. With the connecting tube open, the apparatus was tilted such that the overflow from O_2 just ceased. The tilt was then decreased such that water just trickled from O_2. Under these conditions, the hydrostatic force is reduced to 0 and the hydrodynamic force to a minimum.

FIGURE 26 Adjustment of the constant level apparatus as described by a. Davids (1964) and b. Galtsoff (1926).

Dashed line = base level

Dotted line = height of water required to overcome the surface tension effects around the stand pipe rims.

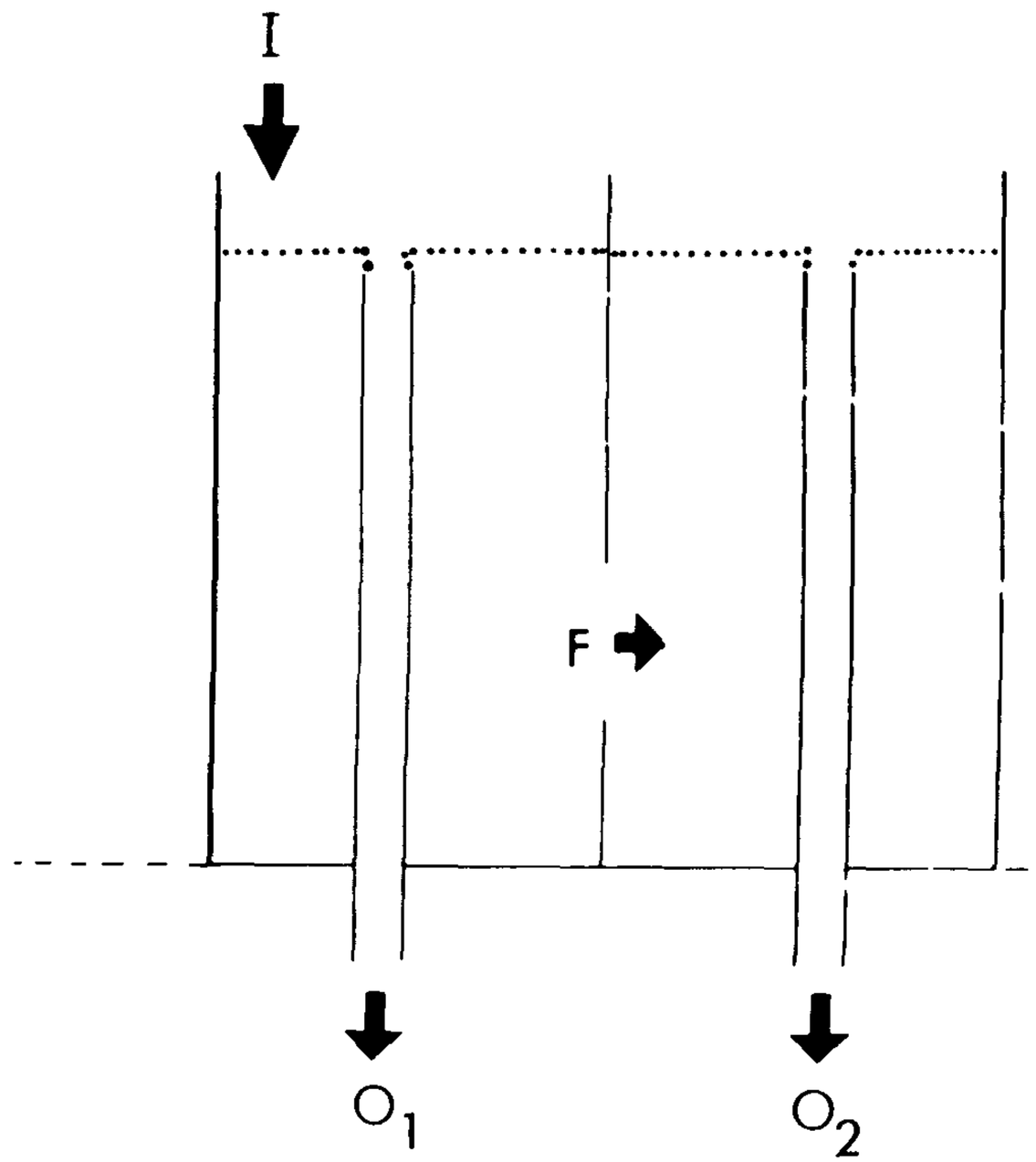
I = inlet

O_1 and O_2 = outlets

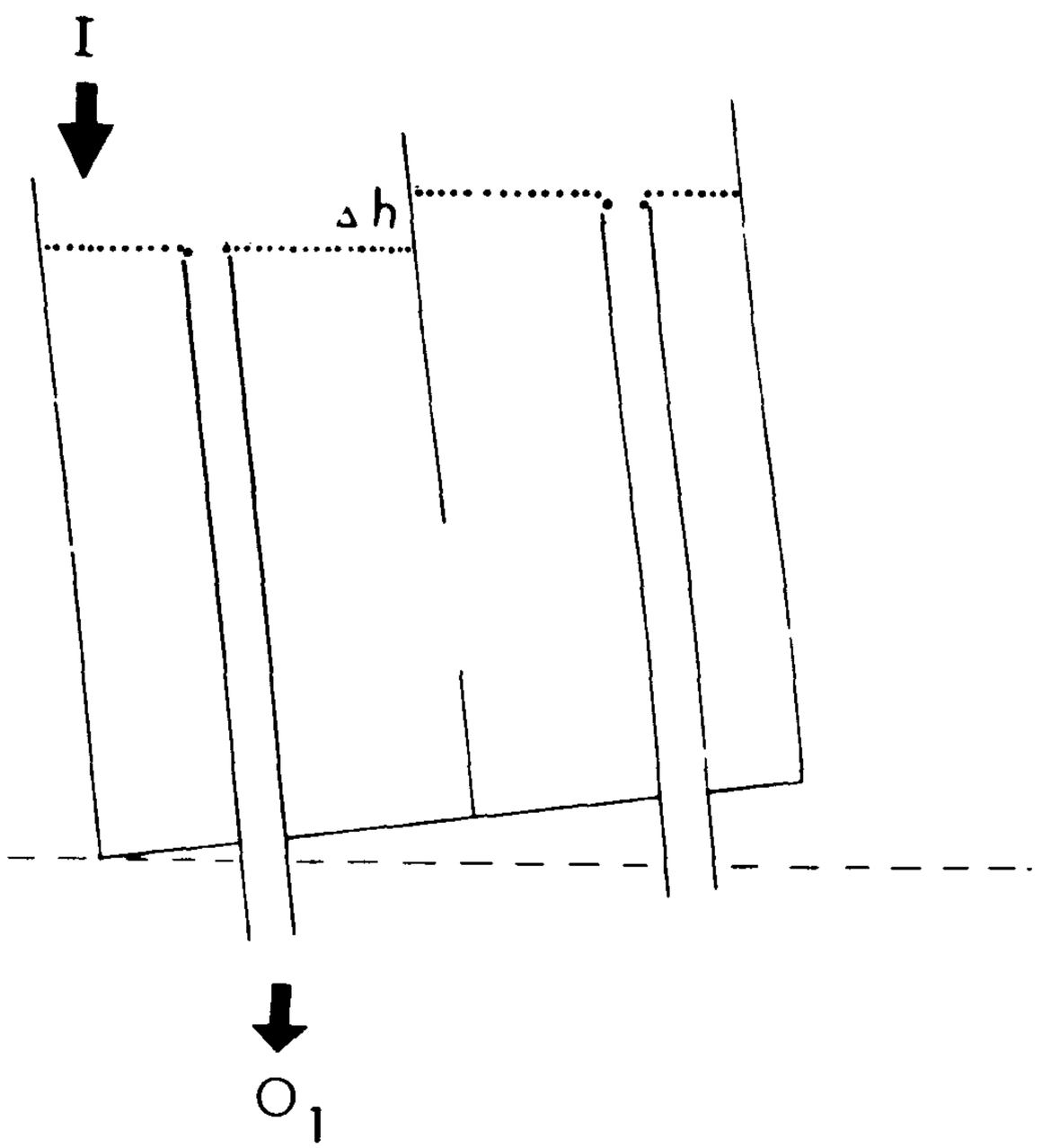
f = dynamic flux

Δh = height difference between water levels.

a.



b.



iii. Experimentation

Animals were left in the apparatus for 2-3 days before experimentation. During this period water in reservoir R was continually replaced with flowing seawater passed through a gravel and glass wool filter. Immediately prior to experiments, the reservoir was isolated from the seawater supply, and concentrated suspensions of Fuller's earth added. The chart recorder was switched on and the flow detector adjusted. Records were made of the pumping activity over 2 hour periods.

RESULTS

Examples of the chart recorder traces produced are given in Fig. 27. Nett pumping rates were calculated from the number of siphons in each 10 minute period. The absolute pumping rate was calculated by dividing the nett rate by the proportion of the 10 minute period spent pumping. The nett rate is generally somewhat less than the absolute rate (Fig. 28) due to occasional stoppages associated with spontaneous squirting. The pumping rates oscillate around a mean in filtered seawater, but in most cases decrease with time when inorganic particulate suspensions are present (Fig. 28).

The average nett pumping rate in filtered seawater is $2,51.h^{-1}$. The rate decreased with increasing suspension load (Fig. 29). The time spent not pumping increased progressively up to $30mg.l^{-1}$ and then declined slightly, but not significantly. This is reflected in the absolute pumping rate which, although progressively decreasing, became much greater than the nett rate with increased particulate load.

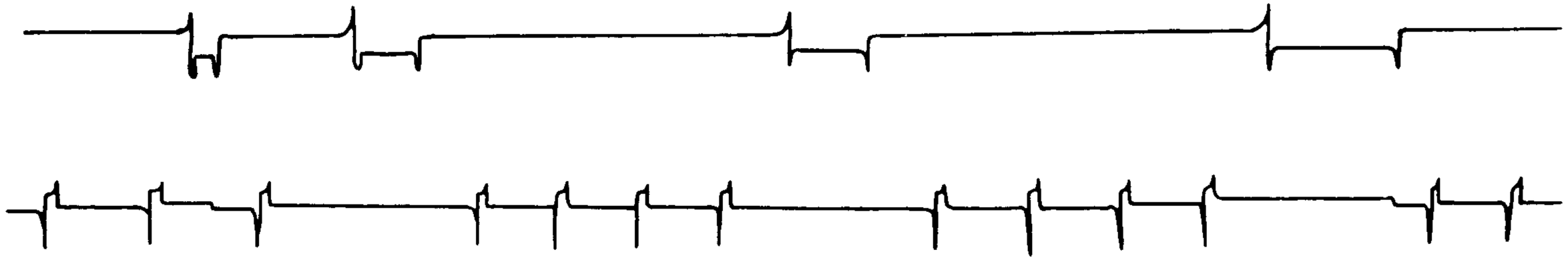
The water in the exhalent chamber remained clear in all experiments, indicating that no inorganic particulate suspensions were passed in the exhaled water from the animals.

FIGURE 27 Examples of records of pumping.

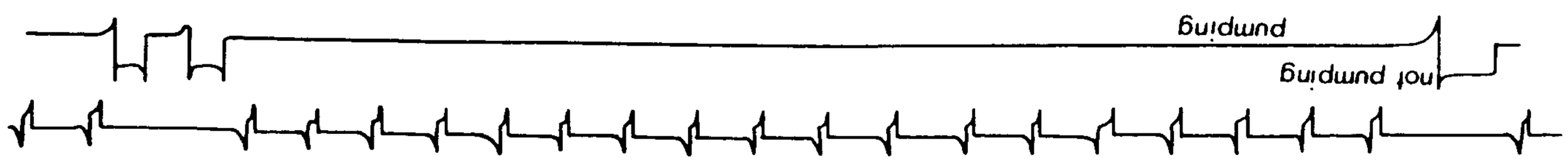
a. in filtered seawater

b. in 15 mg.l^{-1} Fuller's earth

Upper trace from periodic siphon, lower trace from flow detector.



b.



a.

FIGURE 28 Absolute (solid line) and Nett (dotted line) pumping rates ($l.h^{-1}$) of four specimens of A. mentula exposed to suspensions of Fuller's earth. Percentage time not pumping is also shown (%T).

a) filtered seawater

b) $15mg.l^{-1}$

c) $30mg.l^{-1}$

d) $60mg.l^{-1}$

e) $105mg.l^{-1}$.

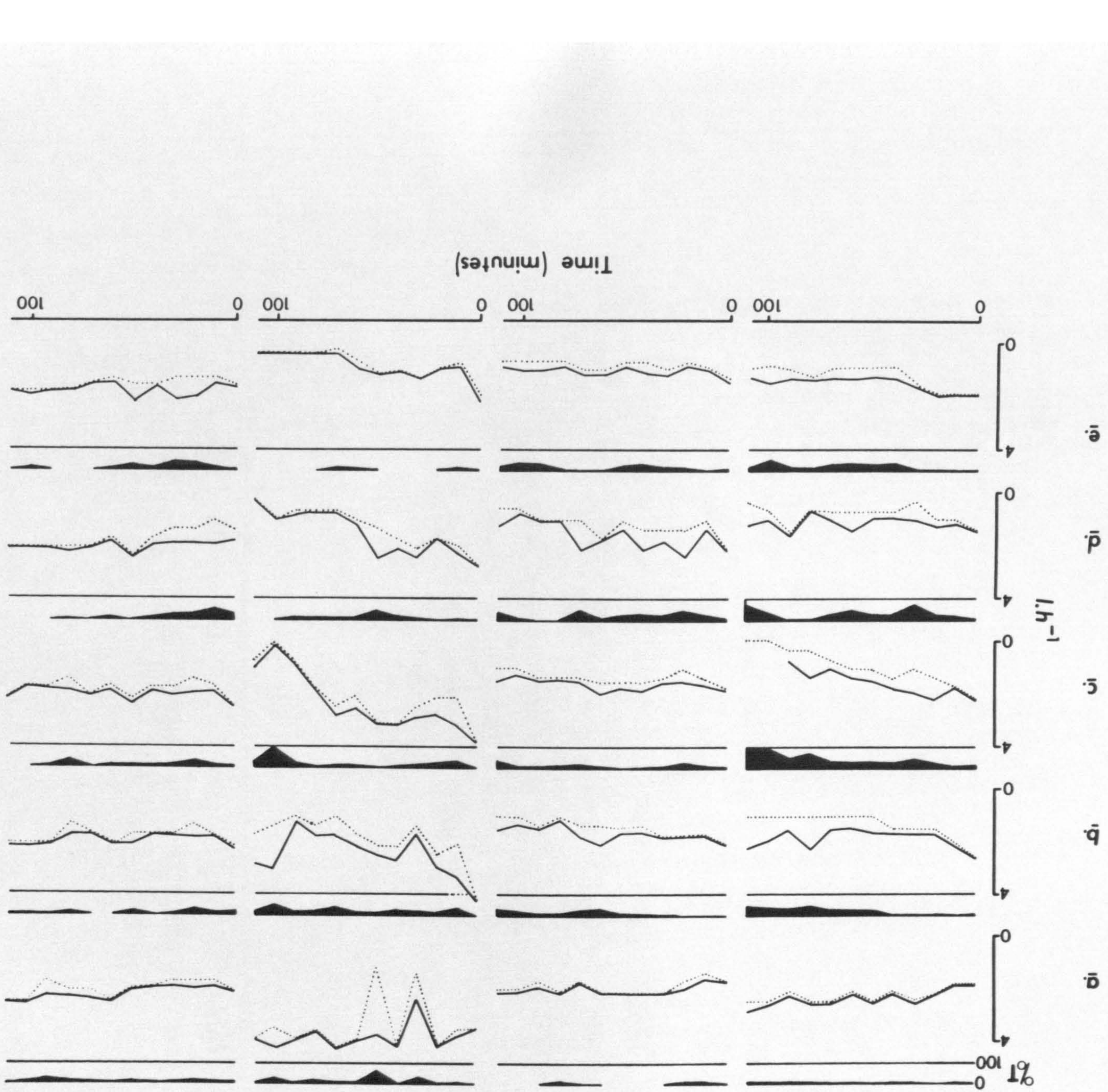

%T
100
0
q.
b.
c.
d.
e.
4
0
Time (minutes)
0
100

FIGURE 29 Mean pumping rates ($l.h^{-1}$) (with standard deviations) of A. mentula exposed to suspensions of Fuller's earth ($mg.l^{-1}$).

● = absolute pumping rate

○ = nett pumping rate

☆ = percent time spent not pumping (right hand scale).

l.h⁻¹
0
1
2
3
0
15
30
60
90
105
mg.l⁻¹
0
%
30

DISCUSSION

There have been no investigations of the pumping rate of ascidians using a constant level apparatus. A few authors, however, have measured pumping rate with other direct methods. Hecht (1916) inserted a glass tube into the exhalent siphon of Ascidia atra and timed carmine particles passing through it. He estimated rates of 0,6-8,8 $l.h^{-1}$ depending on animal weight. Hoyle (1953) used two methods involving the insertion of a glass tube into the exhalent siphon of Phallusia mammillata. Hoyle underestimated the pumping rate due to the creation of a hydrostatic back pressure similar to that created when the constant level apparatus is adjusted in the manner described by Galtsoff (1926). Holmes (1973) used the dye method of Coughlan and Ansell (1964) and calculated rates of 0,2-5,8 $l.h^{-1}$ for Styela clava and 0,3-3,2 for Ascidiella aspersa of various sizes and under various conditions of water movement. Fiala-Médioni (1978a,b,c) calculated pumping rates for various ascidians using hot film thermistors. She found a mean rate of 1236$ml.h^{-1}$ for A. mentula in situ., almost half the rate found in the present study. The animals used in this study, however, were chosen as the largest available. The adjustment of the apparatus must also exaggerate the pumping rate to a small degree. The aforementioned authors have calculated only absolute pumping rates and do not take account of stoppages due to squirting. Fiala-Médioni (1978b) found irregular rates oscillating around a mean with a few brief interruptions due to squirting, similar to the present results at low concentrations of suspended particulates. She used a concentration of $2x10^{7}$ cells l^{-1} monochrysis lutheri. The conditions of these experiments were identical to those used for the calculation of filtration rates and Fiala-Médioni compared the two rates. Pumping rate was consistently higher than filtration rate leading her

to conclude that the filtration efficiencies of Ciona intestinalis, P. mammillata and Styela plicata were between 65 and 90%. In the present study, no inorganic particulates were observed to pass into the exhalent chamber of the apparatus, and it must be concluded that for A. mentula the filtration efficiency is approaching 100%. Randlóv and Riisgård (1979) found retention efficiencies of 100% for C. intestinalis at particle sizes of 2-3µm and above. Filtration rate is a function of the nett pumping rate and is hence likely to be less than the absolute pumping rate. The filtration efficiencies found by Fiala-Medioni could thus be an underestimate. M. lutheri, though, has a size range of 2,5-5µm (Móhlenberg and Riisgård, 1978), so that a small percentage might be expected to escape the ascidian filter.

The pumping rate of P. mammillata becomes more variable and decreases slightly with high concentrations of micro-organisms (Fiala-Médioni, 1979a) in agreement with the decrease in pumping rate found for A. mentula. Similar reductions in rate of pumping were found by Loosanoff and Engle (1947) and Davids (1964) with lamellibranch bivalve molluscs.

The time spent not pumping increases with increasing particulate concentrations up to 30 $mg.l^{-1}$. This result is in agreement with results from Chapter 3. A further increase in time loss is not seen, however, at concentrations above 30 $mg.l^{-1}$, as might be expected from the results of Chapter 3. The pumping rates are much lower at these concentrations, often dipping below 0,5 $l.h^{-1}$. At these low rates the flow detector becomes inaccurate and it is suspected that the time loss is, in reality, higher than that recorded.

The reduction in mean pumping rate (which is proportional to filtration rate) is a function of both the loss of time spent pumping and the reduction in absolute pumping rate. This explains the discrepancies found between the results of Chapters 1 and 3 (summarised in

Table 14). The mechanism of this reduction in absolute filtration rate was not investigated. The partial blocking of the mucus filter by the inorganic particles must account, at least in part, for this reduction. It is possible in addition that A. mentula can control the rate of beating of the stigmatal cilia. This latter possibility seems unlikely however, as it would be energetically more efficient to reduce the rate of filtration solely by this method rather than to increase the rate of squirting.

CHAPTER 5

"Some aspects of the scatology of the ascidians Ciona intestinalis (L.) and Ascidiella scabra (Müller) with special reference to the concentration of inorganic particles suspended in the water".

INTRODUCTION

The shape and composition of faecal pellets have been shown to vary with the quantity and quality of the suspensions used as food by bivalve molluscs (reviewed by Arakawa, 1970). Little information, however, is available concerning the scatology of ascidians. Peters (1966) noted that the strip of mucus coming from the branchial sac is sometimes transported through the intestine as a rather straight band and is sometimes coiled up between membranes which are wrapped loosely around these remarkably flat coils. Peters suggested that this change may be associated with variations in ingestion rate, but there have been no studies using different concentrations of particulate suspensions to investigate this possibility.

Up to satiation point, the ingestion rates of C. intestinalis and A. scabra are positively correlated with suspension concentration. Further increases in concentration cause no further increases in ingestion rate (see Chapters 1 and 2). This phenomenon is likely to be reflected in defaecation rate when ascidians feed on largely indigestible inorganic particulate suspensions. So a study of defaecation rate provides a useful test for the model suggested in Chapter 2.

A. scabra (mud) only was used, A. scabra (Fucus) being smaller, produce small quantities of faeces that would be difficult to weigh.

MATERIAL AND METHODS

Six ascidians were held in a 15 litre tank fitted with two airlifts, constructed from inverted glass funnels and aquarium airstones, positioned 0,5cm off the bottom of the tank. Algal cultures and suspensions of Fuller's earth were pumped into the tank at 100 ml.h^{-1} (Schuco multi-mini pump) from separate 5l round-bottomed flasks. The suspensions in the flasks were agitated by bubbling air into them. The suspension densities of the algal cells and Fuller's earth in the flasks were adjusted such that the required densities were maintained in the experimental tank. The experimental tank was placed in a waterbath and the temperature maintained at 12-15^{o}C.

Faecal strings were collected using a wide-mouthed pipette and allowed to settle in a small beaker. The faeces from the six ascidians were pooled. The supernatant seawater was pipetted out and the faeces washed with distilled water three times. The faeces were then transferred to preweighed crucibles and dried for 24 hours at 105^{o}C before weighing. They were then ashed for 24 hours at 450^{o}C and reweighed.

Faeces were collected weekly in the experiments with Dunaliella salina. In the experiments using Tetraselmis seucica, faeces were collected every 24 hours for a period of 72 hours. Means and standard deviations were taken from the 3 collections.

RESULTS

a) The morphology of the faeces

The faecal pellets of C. intestinalis are, in the terminology of Arakawa (1970), ungrooved crescentic ribbons. This form is best illustrated in Plate 1, for C. intestinalis feeding at 10^5 cells.l^{-1}. The appearance of the faeces changed with increasing suspension load in the water, the crescentic ribbon shape remained essentially unchanged however. The faeces consisted of the food cord (s in Plate 1) thrown back and forth in a sinous manner within a crescentic peritrophic membrane (p). With increasing suspension load the food cord (s) swelled and the sinous folds closed together such that at 10^7 cells l^{-1} the individual folds were indistinguishable. A similar phenomenon was found using inorganic particulate suspensions.

The faecal pellets of A. scabra were very similar to those of C. intestinalis and are hence not described.

b) Biodeposition by A. scabra and C. intestinalis

The weight of faeces produced increased with increasing unicellular algal concentrations in all cases (Figs. 30-32). The weight further increased when inorganic particulate suspensions were filtered, but remained in most cases constant over the range of algal concentrations added in addition to the inorganic particulates (Figs. 30-32).

When inorganic particulates alone were added to the water, the weight of faeces increased with increasing concentration up to 10-20 mg.l^{-1} and then remained constant (Fig. 33a). The regression of percentage organic content of the faeces against inorganic particulate load (Fig. 33b) was not significantly different from zero (student's t test $p < 0,1$).

The percentage organic content of the faeces of ascidians fed solely on unicellular algae varied with algal concentration. In all cases it was minimal at 10^4 cells l^{-1} (Fig. 34a). When ascidians were fed mixtures of inorganic and organic suspensions this minimum becomes indistinct. The lowest values of percentage organic content were found with C. intestinalis in $9mg.l^{-1}$ Fuller's earth and A. scabra in $45mg.l^{-1}$ Fuller's earth.

c) Occurrence of living phytoplankton cells in the faeces

In most experiments a few living cells of Tetraselmis seucica or Dunaliella salina were found in the faeces. At high algal concentrations, the faeces contained so many live cells as to be coloured bright green. At low concentrations the faeces were brown, this being the only instance in which the colour of the faeces did not reflect the colour of the suspension ingested.

PLATE 1 Faecal pellets of C. intestinalis feeding at various concentrations (cells l^{-1}) of Tetraselmis seucica.

p = peritrophic membrane

s = food cord from stomach.

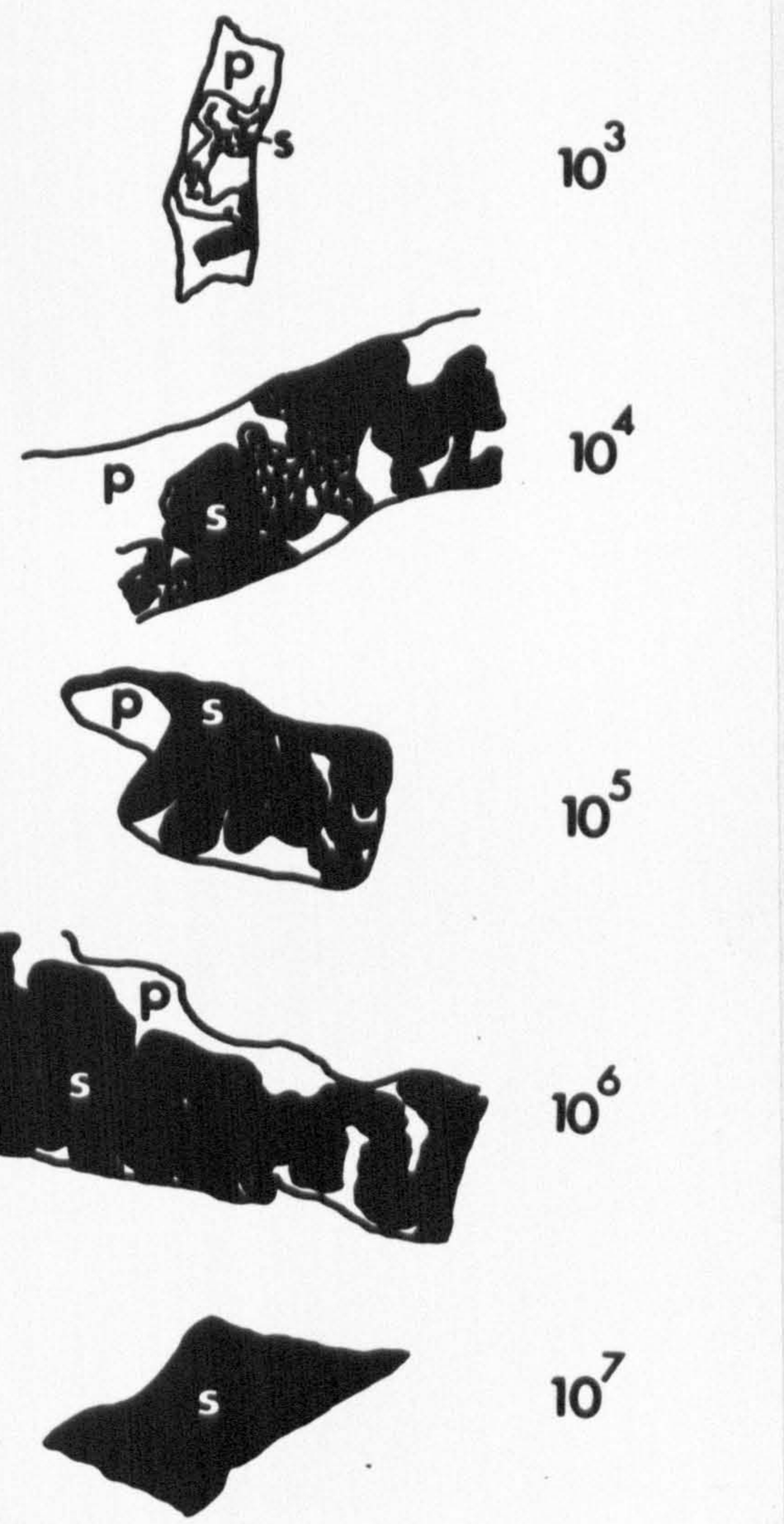

P
S
10^3
P
S
10^4
P
S
10^5
P
S
10^6
S
10^7

FIGURE 30 Defaecation rates of *A. scabra* (mg.24h^{-1} animal^{-1}) at various concentrations of *Dunaliella salina* (cells l^{-1}) and Fuller's earth (mg.l^{-1}).

Shaded portion = ash weight

Unshaded portion = ash free dry weight.

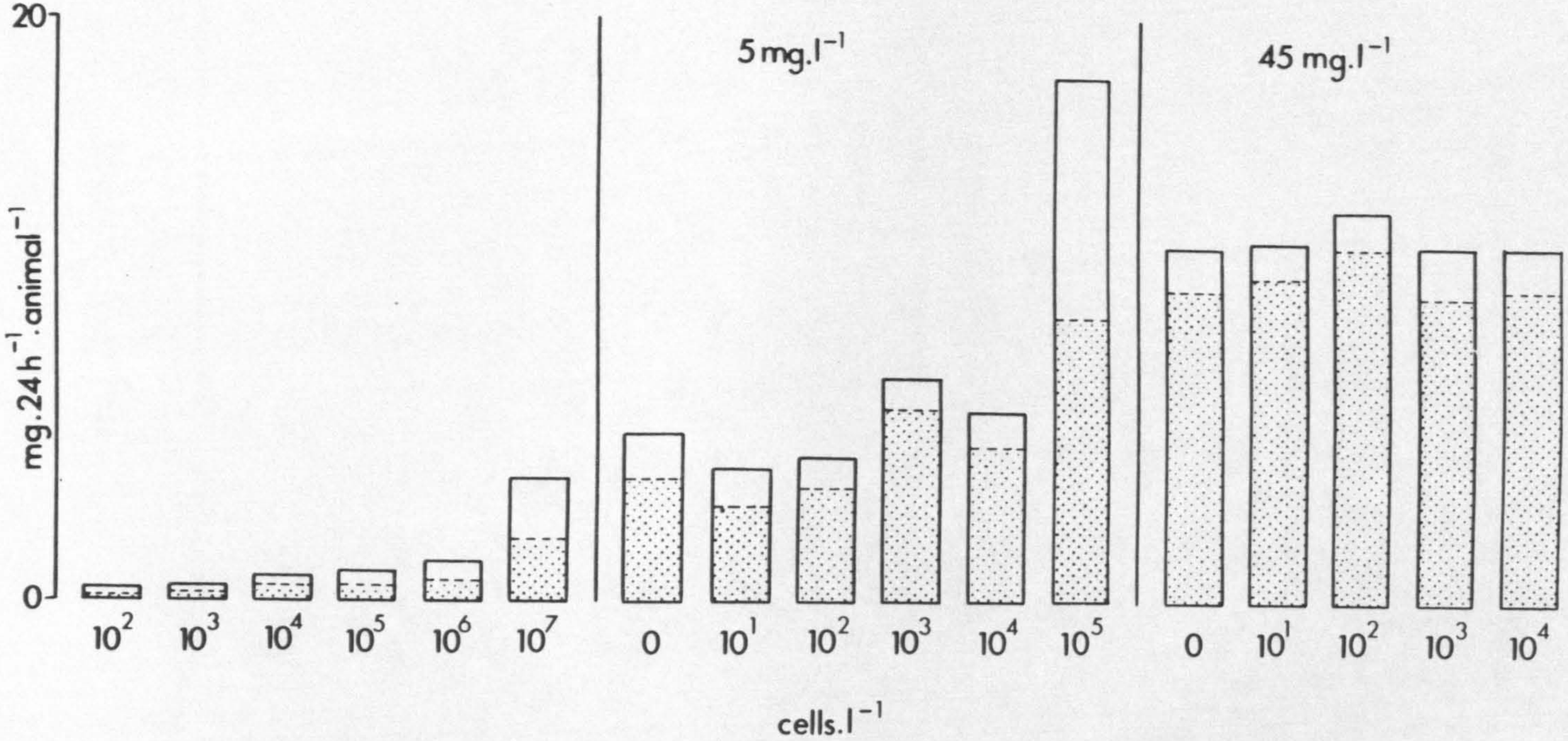

20
0
mg.24 h^{-1}.animal^{-1}
5 mg.l^{-1}
45 mg.l^{-1}
10^2
10^3
10^4
10^5
10^6
10^7
0
10^1
10^2
10^3
10^4
10^5
0
10^1
10^2
10^3
10^4
cells.l^{-1}

FIGURE 31 Defaecation rates of C. intestinalis (mg.24h^{-1} animal^{-1}) at various concentrations of Dunaliella salina (cells l^{-1}) and Fuller's earth (mg.l^{-1}).

Shaded portion = ash weight

Unshaded portion = ash free dry weight.

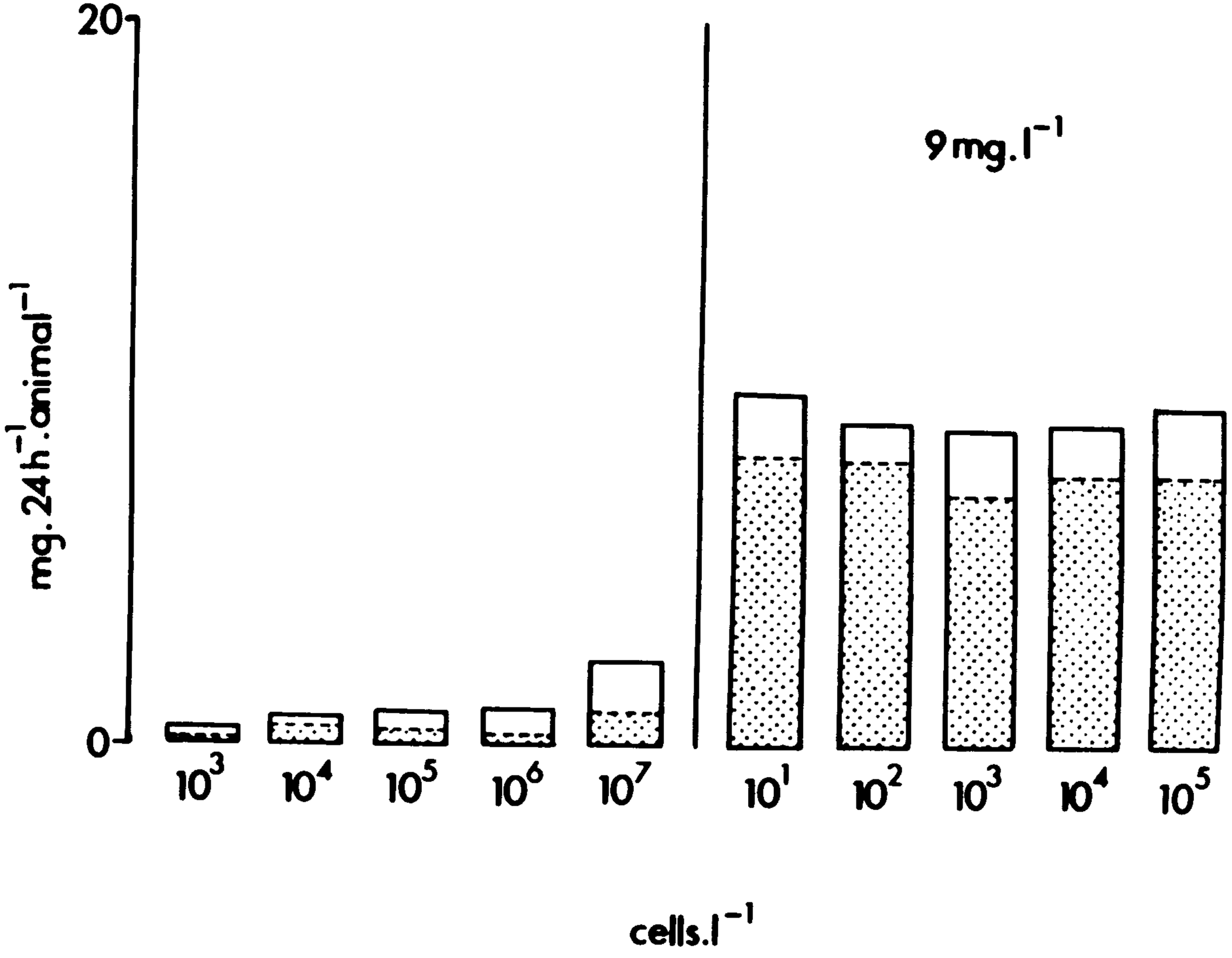

20
0
mg.24h⁻¹.animal⁻¹
9mg.l⁻¹
10³
10⁴
10⁵
10⁶
10⁷
10¹
10²
10³
10⁴
10⁵
cells.l⁻¹

FIGURE 32 Defaecation rates of C. intestinalis ($mg.24h^{-1}$ $animal^{-1}$) (with standard deviations) at various concentrations of Tetraselmis seucica (cells l^{-1}) and Fuller's earth ($mg.l^{-1}$).

Shaded portion = ash weight

Unshaded portion = ash free dry weight.

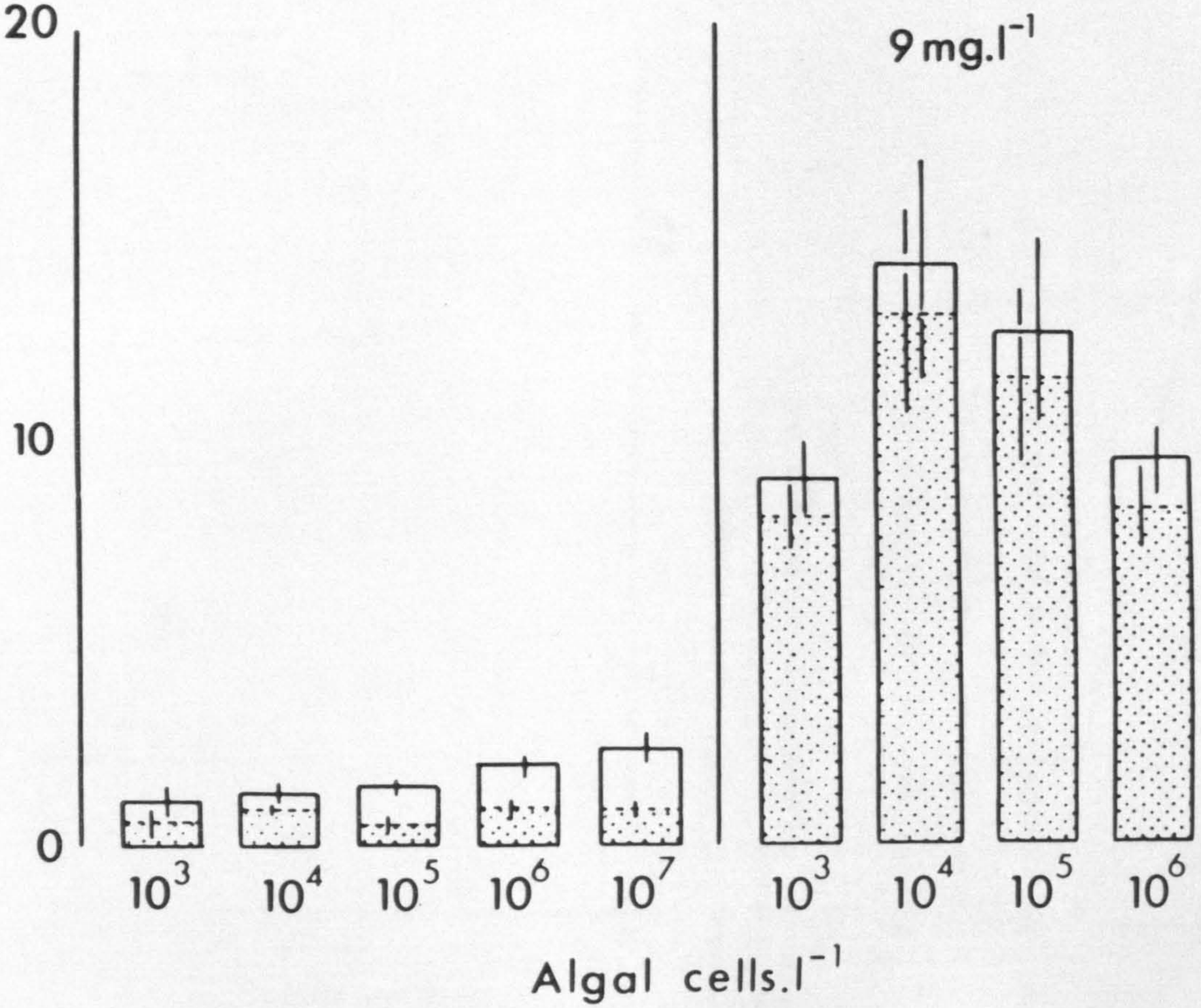

20
10
0
Faeces wt (mg)
9 mg.l⁻¹
10³
10⁴
10⁵
10⁶
10⁷
10³
10⁴
10⁵
10⁶
Algal cells.l⁻¹

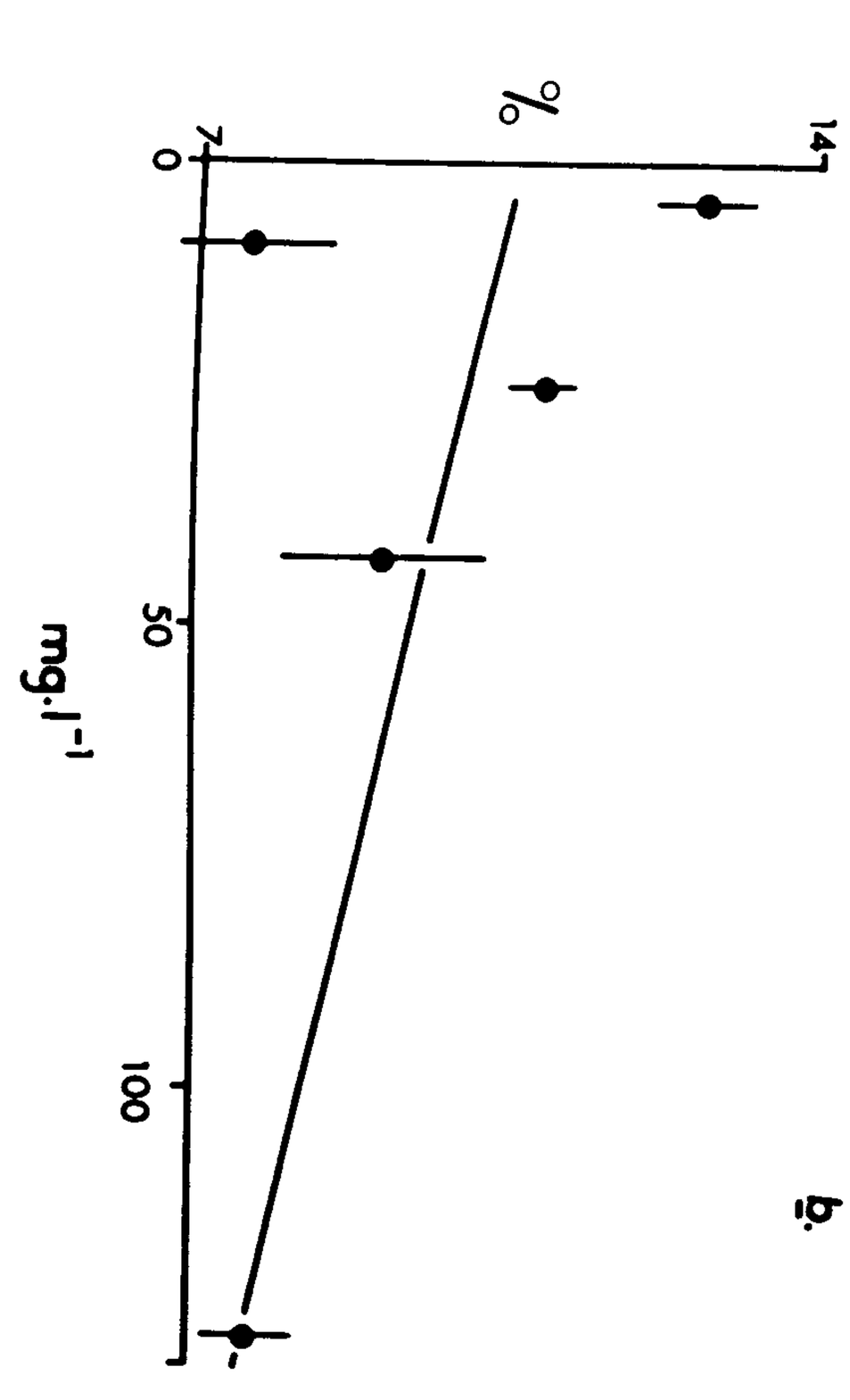
FIGURE 33

a. Defaecation rates (mg.24h^{-1} animal^{-1}), with standard deviations, of C. intestinalis exposed to suspensions of Fuller's earth (mg.l^{-1}).

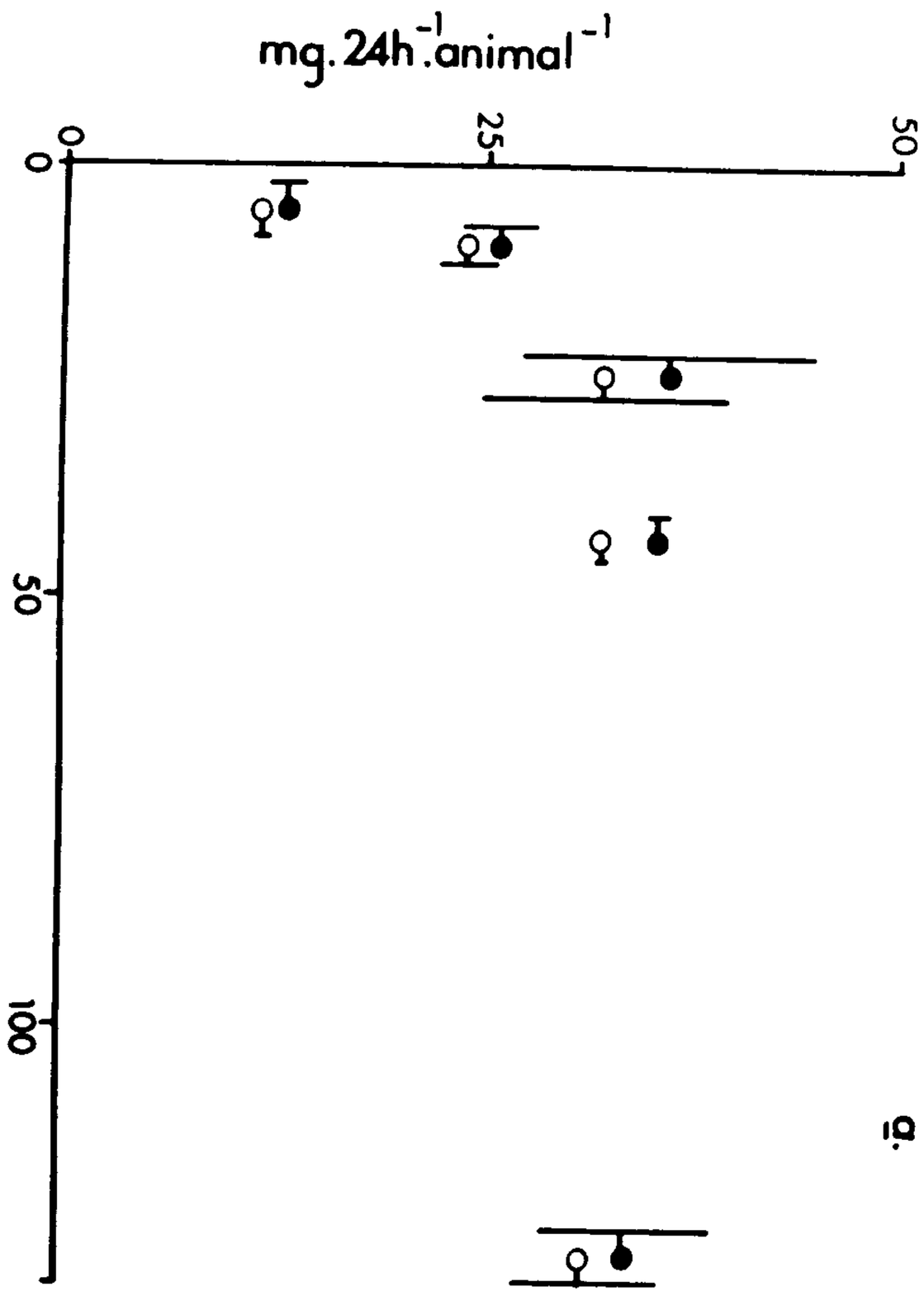
Filled circles = dry weight

Open circles = ash weight

b. Percentage organic content of faeces (with standard deviations).



a)



b)

FIGURE 34 Percentage organic content of faeces collected from ascidians.

a. ○= A. scabra (D. salina)

▲= C. intestinalis (D. salina)

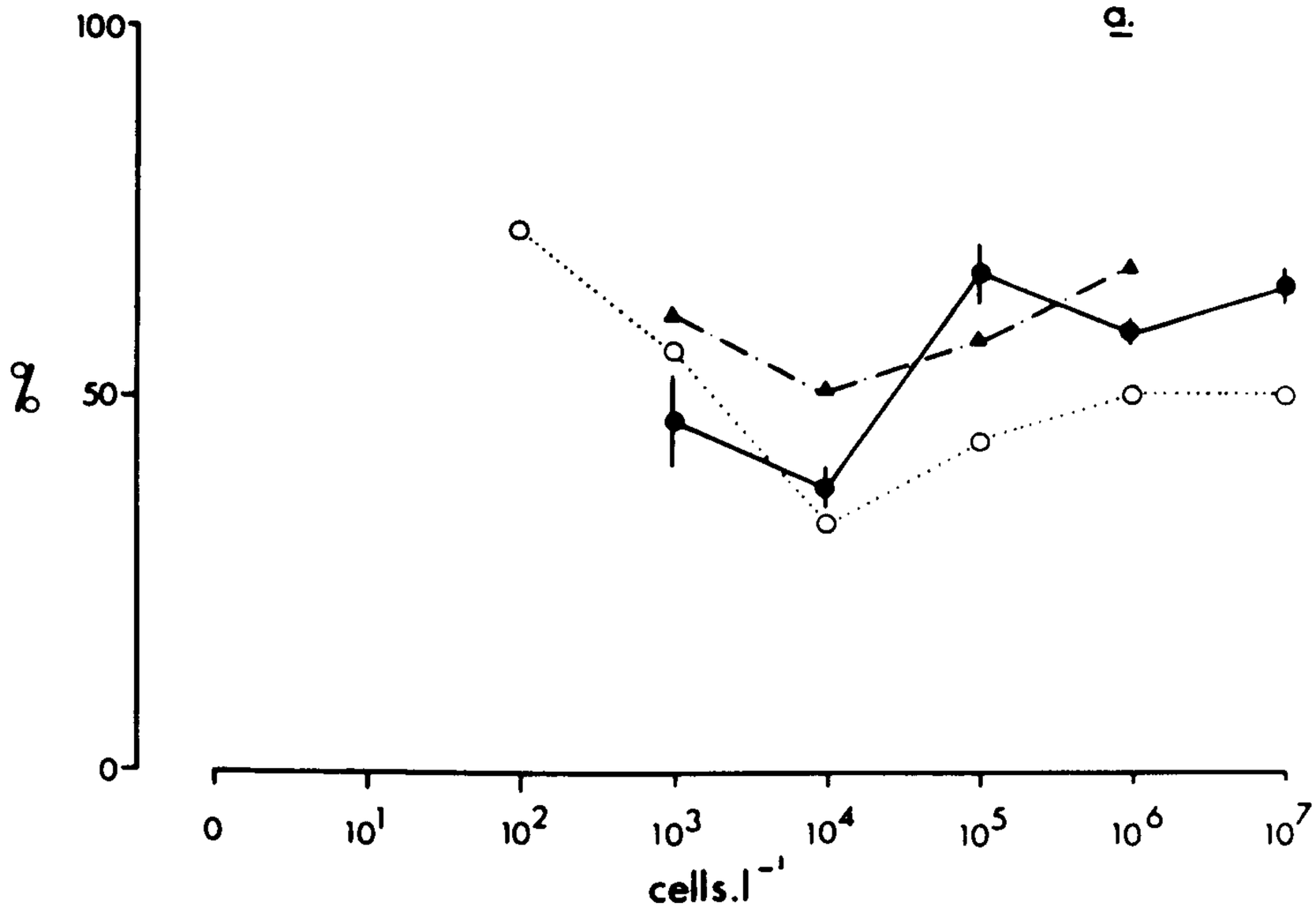
●= C. intestinalis (T. seucica)

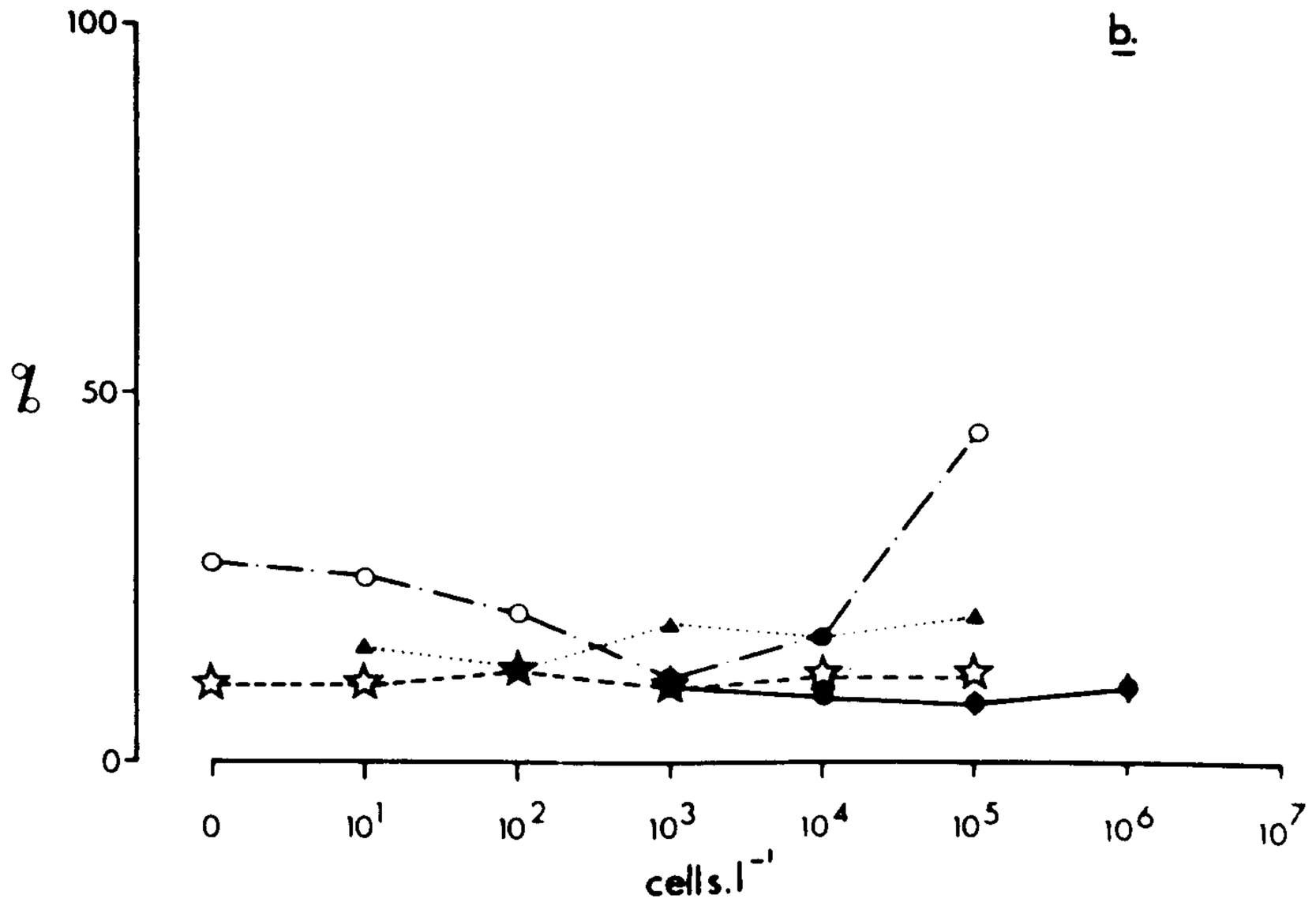
b. ○= A. scabra (D. salina, 5 mg.l^{-1} Fuller's earth)

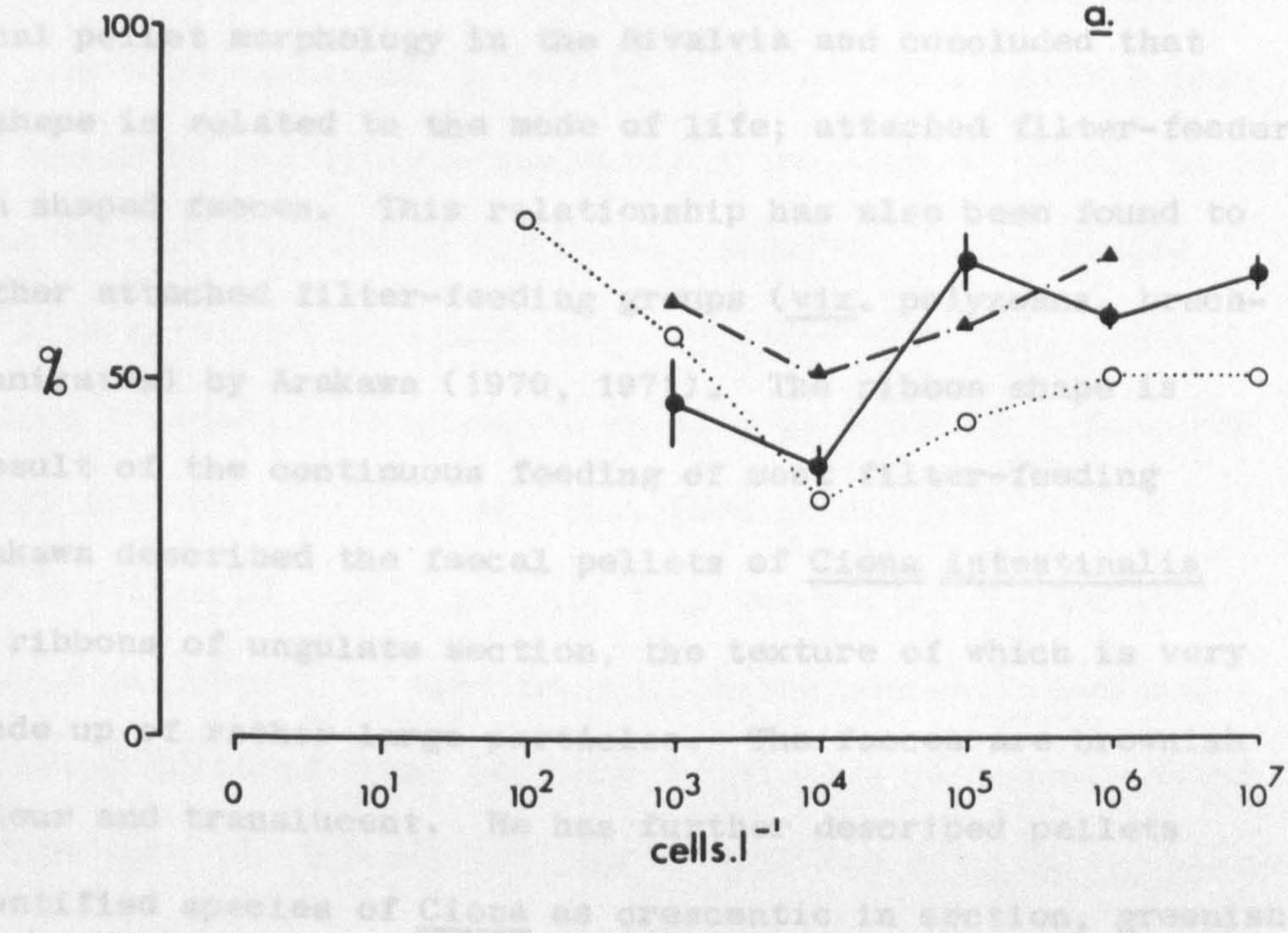
▲= C. intestinalis (D. salina, 5 mg.l^{-1} Fuller's earth)

●= C. intestinalis (T. seucica, 9 mg.l^{-1} Fuller's earth)

☆= A. scabra (D. salina, 45 mg.l^{-1} Fuller's earth).


a.
100
%
50
0
0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
cells.l^{-1}


b.
100
%
50
0
0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
cells.l^{-1}


a.
100
%
50
0
0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
cells.l^{-1}
b.
100
%
50
0
0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
cells.l^{-1}

DISCUSSION

The shape of the faecal pellets of bivalve molluscs has been shown to differ between species (Moore, 1931a,b,c) to such an extent that many species may be identified solely from their faeces. Arakawa (1970) reviewed faecal pellet morphology in the Bivalvia and concluded that the general shape is related to the mode of life; attached filter-feeders having ribbon shaped faeces. This relationship has also been found to be true of other attached filter-feeding groups (*viz.* polyzoans, brachiopods and tunicates) by Arakawa (1970, 1971). The ribbon shape is possibly a result of the continuous feeding of most filter-feeding animals. Arakawa described the faecal pellets of *Ciona intestinalis* as ungrooved ribbons of ungulate section, the texture of which is very coarse and made up of rather large particles. The faeces are brownish yellow in colour and translucent. He has further described pellets from an unidentified species of *Ciona* as crescentic in section, greenish brown in colour, very soft and loose in consistency, usually being shed in very short pieces. The existence of a species of *Ciona* other than *C. intestinalis* is a matter of debate (Dr. R. Millar, pers. comm.). The morphology and colour of *C. intestinalis* can, however, change dramatically from one locality to another. One might tentatively suggest, therefore, that the two species quoted by Arakawa, are one and the same. The differences in colour and texture of the faeces, found by Arakawa, are remarkably similar to the changes found with different concentrations of unicellular algae used as food in the present study. The pellets described for *C. intestinalis* are similar to those found in this study at 10^3 and 10^4 cells.l^{-1}, whilst those described for *Ciona* sp. are similar to those found at 10^7 cells.l^{-1}. At 10^3 cells.l^{-1} the faeces of *C. intestinalis* are brownish yellow in colour due, presumably,

to a large percentage of the algal cells being digested. The faeces are also translucent, as most of the clear peritrophic membrane is not filled by the sinous folds of the food cord. At high concentrations of algal cells the faeces become opaque and greener due to a higher percentage of cells passing through the gut undamaged. These feacal pellets are very soft and break up easily. Similar full pellets from animals fed inorganic particulate suspensions are less brittle. It is probable that the brittleness of the green faeces is caused by the movement of live algal cells within them (see also Hildreth, 1980). The change from crescentic to ungulate section is possibly a function of the smaller size of the Ciona sp. described by Arakawa.

The form and type of faecal pellets from one particular animal have been found to depend, to some degree, on the concentration and type of particles filtered from the water by bivalve molluscs. Moore (1931, b) found that the typical pellets of well fed Mytilus edulis were ribbon shaped and bicrescentic in section, whilst those of starved animals were thin and fragile and often atypical in shape. Hildreth (1980) also recorded that faecal pellets were grooved at low concentrations, but became somewhat cylindrical at higher concentrations. According to the observations of Dinamani (1969) the form of the faecal ribbons of Mytilus and Cardium is dependent upon (a) the time the ingested material is retained within the gut, (b) the type of material ingested and (c) its rate of passage through the gut. Changes in the colour of the faeces dependent upon the time the ingested material is retained within the gut have also been reported by Allen (1962) and Van Weel (1961). The feeding mechanism is more complicated in bivalves, however, due to rejection currents in the stomach and the passage of a portion of the food into the digestive gland. The form of the faecal pellets of C. intestinalis and A. scabra is dependent only upon the ambient particle

concentration, particle quality changing only the colour. The external morphology of the faecal pellets remained in all instances relatively constant. Similarly, Reeve (1963c) reported little change in the shape and dimensions of the faecal pellets of Artemia when the animals had been feeding in different suspensions. Furthermore he found that starved animals produced pale membranous faeces of similar shape to normal faeces and at the same rate as fed animals. These faeces were often mottled with what appeared to be colonies of bacteria. Similar observations were found with C. intestinalis in the present study.

The morphology of the faecal pellets is best understood by consideration of the passage of food through the gut. The food cord produced by the pharynx (see General Introduction) is drawn continuously into the oesophagus (Plough and Jones, 1939). The cilia of the oesophagus impart torsion on the food cord (Millar, 1953) such that the food cord folds upon itself in the stomach (Plough and Jones, 1939; Berrill, 1950; Goodbody, 1974). According to Yonge (1936) and Morton (1960) acidity in the stomach causes a reduction in the viscosity of the food cord such that it becomes softer and more diffuse, and in C. intestinalis is often not discretely recognisable (Morton, 1960). The folding of the cord in the stomach, however, suggests that the mucus envelope remains intact with its viscosity unchanged (Goodbody, 1974). My own observations would support the latter view. At high suspension loads the folds of the cord become so tightly packed that they are not discretely recognisable. In the views of MacGinitie (1939) and Goodbody (1974) any change of viscosity must occur at the junction of the stomach and intestine. At this junction there is a ciliated ring differentiated from the rest of the gut (Millar, 1953; Croxall, 1971). Goodbody regarded this ring as more important in assisting the passage of a semi-fluid material than in pulling the cord into the intestine. If this

is the case, it is possible that the torsion imparted by the oesophagus is responsible for the sinuous folding of the food cord on entering the intestine. This folding has previously been reported by several authors (Morton, 1960; Croxall, 1971; Goodbody, 1974; Plough, 1978 in his Fig. 24). In C. intestinalis the intestinal lumen is crescentic in shape due to the presence of a prominent typhlosole occupied by sexual ducts (Yonge, 1925). The flattening of the intestine provides a greater surface area of resorbtive cells for a given volume of lumen. The crescentic shape is due, perhaps, to the proximity of the intestine to the sexual ducts. The folding back and forth of the food cord provides an increased gut passage time (and hence time available for digestion and absorption) without necessitating an unduly long intestine or a reduction in the rate that the pharyngeal mucus cord is passed into the gut. The intestine secretes a mucous peritrophic membrane around the folded food cord completing the crescentic ribbon that will be defaecated. Peters (1966) has reported that this peritrophic membrane is rich in chitin. The mucus secreted by the mid-intestine is rich in carbohydrate-protein complexes (Thomas, 1970a,b; Croxall, 1971) whereas that secreted by the oesophagus is predominantly composed of acid mucopolysaccharides (Relini-Orsi, 1969; Thomas, 1970a,b; Croxall, 1971). Chitin is a carbohydrate with a tendency to associate with proteins and inorganic salts. It might constitute one of the carbohydrate-protein complexes reported by Thomas and Croxall. The cells at the pyloric end of the intestine are mainly resorbtive cells; giving way to a predominance of cells containing mucus droplets towards the rectal end (Tunas, 1977). It would seem likely, therefore, that the peritrophic membrane is laid down to the greatest extent in the latter portion of the intestine and the rectum. The peritrophic membrane gives the faeces a firm binding. This is important for filter-feeding animals

since the discharge of loose faeces could foul approaching currents (Morton, 1960). The proximity of the folds of the food cord in the intestine (and hence in the faeces) is shown to be a function of the ingestion rate, verifying the suggestion of Peters (1966). The food cord, however, never passes without folding, as is suggested by Peters, at low concentrations. The drawings of Mareile Fenner (Plough, 1978, Plate I) show C. intestinalis with what would appear to be satiated and nearly satiated faecal pellets in the recta.

The passage of viable phytoplankton cells through the guts of planktonic herbivores is well documented (Blegvad, 1915; Allen, 1921; Harvey et al., 1935; Coe and Fox, 1942; Fox and Coe, 1943; Coe and Fox, 1944; Coe, 1945, 1947, 1948; Loosanoff and Engle, 1947; Verwey, 1952; Marshall and Orr, 1955; Dinamani, 1969; Porter, 1973, 1975). Nelson (1933) is of the opinion that this phenomenon occurs in the oyster as a result of an incomplete separation of food particles from undigestible material that is inherent to the feeding mechanism. This seems unlikely as the phenomenon also occurs when animals are fed pure cultures in the laboratory. The feeding process is complicated in bivalve molluscs, however, and portions of food taken in at one particular time may be egested at different times (Allen, 1962; Dinamani, 1969; Foster-Smith, 1975b) and hence be subjected to different digestive efficiencies. It has been suggested that certain unicellular algae possess a resistance to digestive enzymes. Hildreth (1980) found that Tetraselmis seucica (a unicellular flagellate) was unchanged by the gut of Mytilus edulis, whilst the diatom Thallassiosira pseudonoma, at a similar high concentration, was broken up. Coe and Fox (1944) and Coe (1948) have suggested that the cellulase activity is inadequate in bivalves, as dinoflagellates and other phytoplankton with cellulose walls pass through the canal undamaged. It is also possible that algae

with a gelatinous cell wall are more resistant to the action of the style than diatoms with a delicate frustrule. Porter (1973, 1975) found that cells encased in a thick gelatinous sheath were the most resistant to breakdown by zooplankton. The possibility that suspension density (or to be more exact, ingestion rate) might influence this phenomenon has been indicated by Marshall and Orr (1955), with their finding of unchanged cells in the faeces of Calanus finmarchicus at high feeding levels, and Harvey et al., (1935) who reported its occurrence in natural populations of copepods during the spring phytoplankton bloom. Currie (1962) states that "findings of a rapid degradation of ingested chlorophyll may seem to conflict with evidence of the passage of living phytoplankton cells through the guts of planktonic herbivores, but no doubt this can be reconciled in the feeding rate". The phenomenon has also been ascribed to an excess of food by Fox and Coe (1943), and Allen (1921) has noted the simultaneous occurrence of digested and undigested green diatom cells, suggesting that bivalves are capable of digesting these cells. It would seem probable, as suggested by Verwey (1952), that it is a combination of both factors. The fragility of the green faecal pellets might enable the viable, and partially digested, cells to become re-available for ingestion by the same or neighbouring animals (c.f. Mook, 1981).

The quantity of faecal pellets produced by Calanus finmarchicus has been shown by Marshall and Orr (1955) to increase to a maximum, and then remain constant, with increasing suspension load. This result is similar to that found for C. intestinalis fed solely on Fuller's earth (Fig. 33a). Since faecal pellets containing Fuller's earth are heavier than those containing the remains of unicellular flagellates, the results of experiments involving mixed suspensions are difficult to analyse on a similar basis. When the quantity of

inorganic particulate material greatly excedes that of the algal cells (i.e. at 9 and 45 $mg.l^{-1}$) defaecation rate remains, to a large degree, constant (Figs. 30-32). These results therefore, accord well with those for ingestion rates (Chapters 1 and 2). Arakawa (1970) has pointed out a discrepancy in the results from bivalve molluscs. Ota (1959; cited by Arakawa, 1970) demonstrated that, during summer and autumn, the faecal and pseudofaecal production of Pinctada martensii was correlated to the ambient suspension load. Similar results were obtained in the laboratory by Lund (1957), with both faecal and pseudofaecal volumes being positively correlated with suspension load. Winter (1978) has suggested that pseudofaecal production commences only once the gut is satiated. Increases in faecal production whilst pseudofaeces are being produced are, hence, difficult to account for. Haven and Morales-Alamo (1966, a) have demonstrated that, in Crassostrea virginica, the rate of pseudofaecal production is positively correlated to suspension density, whereas that of faecal production shows no correlation. Similar results for pseudofaecal production have been indicated by Loosanoff and Engle (1947); the latter authors have found, however, that faecal production declines with increasing suspension load. Both Arakawa et al., (1971) and Haven and Morales-Alamo (1966b) have found greater production of faeces by bivalve molluscs during autumn and winter, times of year at which Bayne and Widdows (1978) recorded minimal clearance rates in mussels, correlated with maximal total particulate suspension loads. From these data it would be difficult to deduce with certainty the relationship between bivalve faecal production and ambient seston concentration. Food passage in bivalves is, however, far more complicated than in ascidians.

From the data presented in this study, it is apparent that the rate of production of faeces by ascidians varies over a certain range

of ambient seston concentration. This makes comparison of data between authors more difficult. Blegvad (1915) found rates of 0,59g and 1,2g for 100g tissue weights of C. intestinalis and Phallusia mammillata respectively. For the size of C. intestinalis used in the present study this is equivalent to about 60 and 120mg.animal^{-1}.day^{-1}. Rates of 40 and 80mg.animal^{-1}.day^{-1} have been reported for Molgula manhattensis by Haven and Morales-Alamo (1966a). Weight specific defaecation rates (i.e. weight of faeces produced per diem divided by animal weight) range from 0,1-2,5 for filter-feeders (Haven and Morales-Alamo loc. cit.), M. manhattensis having a rate of 2,3-2,5. Figures calculated from the present study are 0,9-1,4 for A. scabra and 0,9-1,6 for C. intestinalis at maximum defaecation rates. But the results are not strictly comparable. The weight of faeces depends upon the specific density of the suspended particles ingested. Stolidobranchs (such as M. manhattensis) have been shown to have higher weight specific filtration rates than phleobranchs (such as A. scabra and C. intestinalis) by Fiala-Médioni (1974). Perhaps they also have a higher ingestion rate? Arakawa et al., (1971) has reported rates of 31,2mg.animal^{-1}.day^{-1} in December, decreasing to 17,7mg.animal^{-1}.day^{-1} in April for C. intestinalis.

The production of faecal pellets (termed biodeposition by Haven and Morales-Alamo, 1966a) has a profound effect on the physical and chemical characteristics of the sea bottom (Moore, 1931a,b,c; Damas, 1935; Ito and Imai, 1955). Moore found in many cases that up to 40% of the fine material of muds covering the bottom of the Clyde Sea area was consolidated into faecal pellets. Haven and Morales-Alamo (1966, a) pointed out how biodeposition can influence sediment transportation rates and initiate the sedimentation of particles between 1 and 3 µm, and demonstrated the incorporation of biodeposits into the sediment (Haven and Morales-Alamo, 1966b). Ascidians might be expected to have a greater influence on suspended particles of this size range than

bivalve molluscs, as they can filter them more efficiently (Móhlenberg and Riisgård, 1978; Randlóv and Riisgård, 1979). Due to a lack of pseudofaeces production, however, ascidians might be expected to produce less biodeposits than bivalve molluscs.

Increases in the percentage organic content of the faeces above 10^4 cells.l^{-1} ambient concentration might be explained by reduced digestion efficiency, illustrated by a high proportion of viable cells in the faeces. Increases below this level are thought more likely due to the mucus of the food cord and peritrophic membrane constituting a higher proportion of the faecal weight than at higher concentrations.

CHAPTER 6

"Assimilation efficiency and gut residence time in the ascidian *Ciona intestinalis* (L.), as functions of ambient organic and inorganic suspension concentration".

INTRODUCTION

The percentage organic content of the faeces of C. intestinalis and A. scabra fed on Tetraselmis seucica or Dunaliella salina increases as a function of ambient cell concentration, between the concentrations of 10^4 and 10^7 cells.l^{-1}. This is possibly due to a decreased assimilation efficiency, with increases in the percentage organic content at ambient concentrations of less than 10^4 cells.l^{-1} being more likely due to an increasing proportion of mucus in the faeces (see Chapter 5). Further evidence for a reduced assimilation efficiency at high algal concentrations is given by the passage of living cells in the faeces. This phenomenon may be caused by the unsuitability of the algae as food, but Currie (1962) concluded "reports of the excretion of living cells all appear to originate from animals feeding at an excessive rate on dense cultures of algae and the maximum rate of digestion may well be reached before the maximum rate of physical ingestion".

To estimate the assimilation efficiencies of C. intestinalis at various concentrations of the unicellular flagellate Tetraselmis seucica and Fuller's earth, three simple methods were employed. These methods involved neither the use of radioactive markers nor the quantitative collection of faeces.

One aspect of the feeding of an animal, that can influence the degree of digestion and assimilation, is the gut residence time. This was estimated at various concencentrations of algal cells and Fuller's earth, such that the residence times could be compared when there was i) no food, ii) incomplete satiation of the gut with organic and inorganic material, and iii) complete satiation with organic and inorganic material.

MATERIAL AND METHODS

a) Assimilation efficiency

Three methods were employed to estimate assimilation efficiencies at different ambient particle concentrations. In all experiments 3 animals were held at each concentration in 31 jars positioned in a waterbath to maintain the temperature at 15 $\pm$ 1^{0}C. Tetraselmis seucica and suspensions of Fuller's earth were supplied to the jars as described in Chapter 5. An air stone in each jar maintained the particles in suspension.

i. Cell count method

Microscope slides, each with a coverslip, were individually wrapped in aluminium foil. They were dried for 24 hours at 105^{o}C and then left for 8 hours in the balance room before being weighed to 5 decimal places on a chemical balance. Animals were sacrificed after feeding for several days at one particular ambient particle concentration. Small portions of the food cord were taken from the oesophagus and the rectum. The samples were placed on individual weighed slides and total, whole, green cell counts made at 400X magnification. The slides were re-wrapped in the same pieces of foil from which they had been taken. They were dried for 24 hours at 105^{o}C, allowed to equilibrate to the temperature and humidity of the balance room for 8 hours and re-weighed. The percentage assimilation efficiency (As %) was calculated from the equation:-

$$As\ \% = 1 - \frac{R}{O} \cdot 100$$

where O and R are the weight specific cell counts of the food cord in the oesophagus and the rectum respectively. This method is a modification of the technique used by Croxall (1971).

ii. Conover ratio method

This method relies on the assumption that inorganic material is not affected by the digestive processes, the assimilation efficiency thus being a function of the change in percentage organic content, relative to the constant quantity of inorganic material, between the food and the faeces. The percentage assimilation efficiency was calculated from the equation derived by Conover (1966):

$$As\ \% = \frac{(F' - E')}{(1-E')\ (F')}\ 100$$

where F' and E' are the ash-free dry weight; dry weight ratios (i.e. fractions of organic matter) in the food and the faeces respectively.

iii. Maynard and Loosli ratio method

The faeces of ascidians are bound in mucus which is largely organic in nature. Thus, there is an organic component in the faeces additional to that comprising unassimilated food material and a tendency to underestimate the percentage assimilation efficiency when using the Conover ratio. This phenomenon becomes most significant at low feeding levels when mucus forms a large proportion of the faeces (see Chapter 5). For this reason, a third method was chosen that would be less influenced by the presence of mucus in the faeces.

Assimilation efficiency can be calculated by reference of the concentrations of a given nutrient in the food and faeces to that of a physiologically inert material (Maynard and Loosli, 1969), using the equation:

$$\text{As } \% = 100 - \left(100 . \frac{\% \text{ indicator in food}}{\% \text{ indicator in faeces}} \frac{\% \text{ nutrient in faeces}}{\% \text{ nutrient in food}} \right)$$

The physiologically inert compound chromic oxide (Cr_2O_3) has been used by Forster and Gabbott (1971). In the present study, however, the use of such a compound was considered undesirable, as it would alter the total suspension load of the water. The naturally occurring inorganic content of the ingested food, though, was used as the reference material. In order to minimise the influence of mucus in the faeces, protein was used as the nutrient investigated. The protein content of the faeces and the food was measured using the Folin phenol method, described by Lowry et al., (1951). The mucus contains only a small proportion of protein, that was detected neither by Winter (1969; using the biuret technique) nor by Fiala-Médioni (1973; using the Lowry technique).

The equation given by Maynard and Loosli (loc. cit.) is modified to:

$$\text{As } \% = 100 \left[1 - \left(\frac{I_f . P_e}{I_e . P_f} \right) \right]$$

where I_f and I_e are the ratios of inorganic (ash) weight to total dry weight for the food and the faeces respectively; P_f and P_e are the ratios of protein weight to total dry weight for the food and the faeces respectively.

b) Gut residence time

Four individual C. intestinalis were held in separate mesh cages in a 15l tank fitted with two airlifts, constructed from inverted glass funnels and aquarium air stones. Filtered seawater, algal suspensions or Fuller's earth suspensions were supplied to the tank, via a multi-mini pump (Schuco), from a 5l round-bottomed flask, as

described in Chapter 5. At time zero a small aliquot of carmine suspension was added to the tank close to the oral siphons of the animals. The time required for the first carmine stained faecal pellet to be ejected was recorded to the nearest 15 minute period. The experiment was repeated in triplicate for each concentration of suspension investigated.

RESULTS

The assimilation efficiency of C. intestinalis is shown, by all three methods, to be a declining function of the concentration of Tetraselmis seucica between the concentrations of 10^3 and 10^7 cells.l^{-1} (Fig. 35). The assimilation efficiency was found, with the cell count and the Maynard and Loosli ratio methods, to be between 90 and 100%, at the lowest cell concentrations. The Conover method gave a lower value of 72%. At 10^7 cells.l^{-1} all three methods yield values between 25 and 40%. With 9 mg.l^{-1} Fuller's earth suspension, the assimilation efficiency, as estimated by the cell count method, remains more or less constant, over the range of algal concentrations (Fig. 35a). The mean assimilation efficiency under these conditions is the same as that found at 10^7 cells.l^{-1} without Fuller's earth. The results are slightly lower, as estimated by the Conover ratio method (Fig. 35b) especially at the lowest algal cell concentration. There was not enough protein present in the faeces, when 9 mg.l^{-1} Fuller's earth was present, to be estimated reliably.

The gut residence time varies slightly with the degree of satiation (Table 15). Due to the large variations between animals and daily variations between the results for individual animals, however, the gut residence times are not significantly different ($P < 0.05$) (see Table 16).

FIGURE 35 Assimilation efficiencies (As %) of *C. intestinalis* at various concentrations of *T. seucica* (cells l^{-1}).

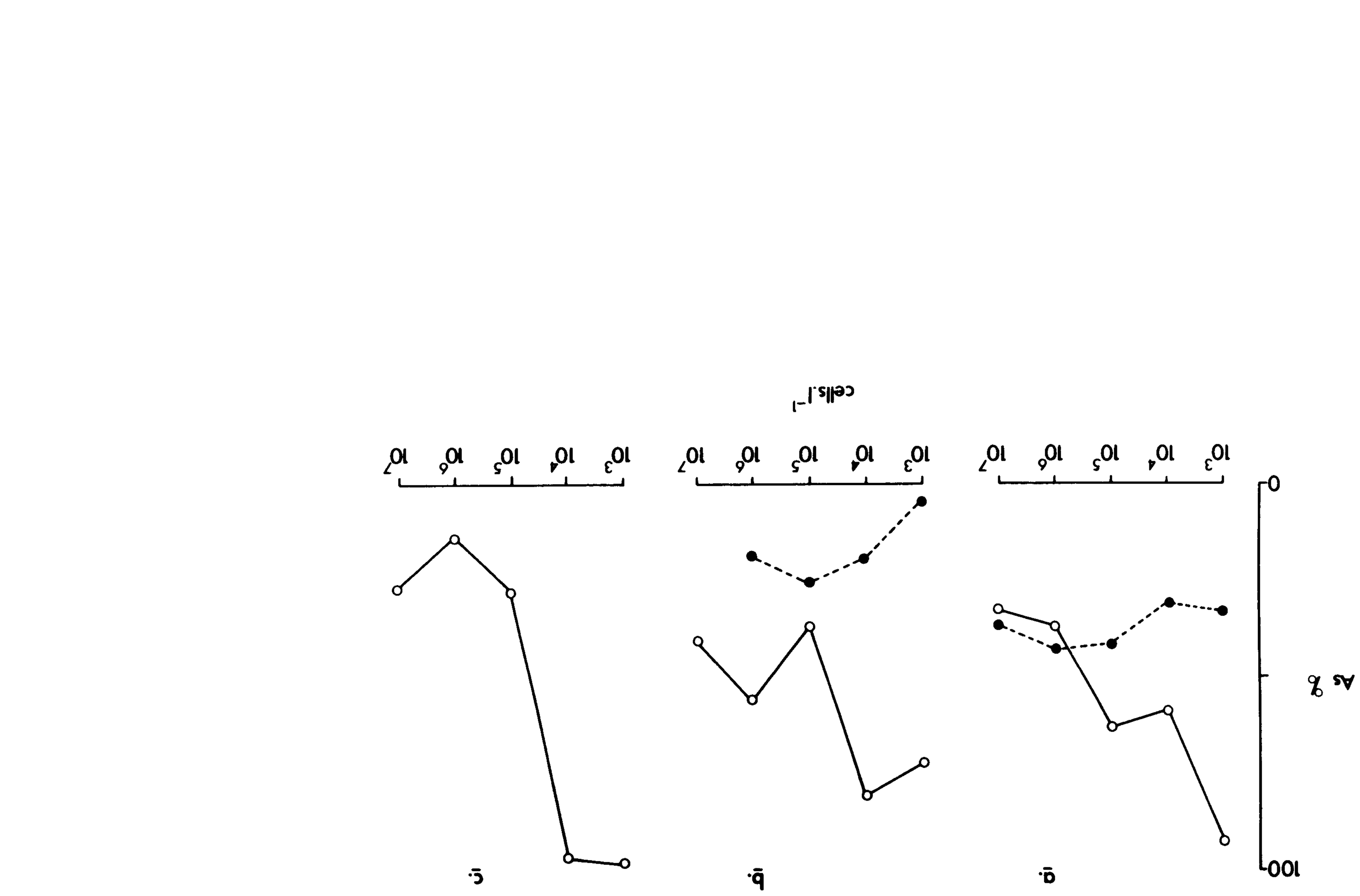
Open circles = algal cells only

Filled circles = algal cells plus 9 $mg.l^{-1}$ Fuller's earth.

a. cell count method

b. Conover ratio method

c. Maynard and Loosli method.


0
100
As %
10^3 10^4 10^5 10^6 10^7
cells.l^{-1}
a.
b.
c.

STATE OF INGESTION	PARTICULATE TYPE	GUT RESIDENCE TIME	STANDARD ERROR
0	-	23,00	1,26
Incomplete satiation	organic	23,50	0,68
	inorganic	22,25	1,27
Complete satiation	organic	20,75	1,01
	inorganic	19,75	2,35

TABLE 15 Gut residence times (hours) of C. intestinalis at 15°C.

SCOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES
Between conditions	56,678	4	14,1695
Residual	753,213	47	16,0258
Total	809,891	51	-

TABLE 16 Anovar of gut residence data.

DISCUSSION

Fiala-Médioni (1973, 1974) has reported high assimilation efficiencies (83-93%) in the ascidians Phallusia mammillata, Clavelina lepadiformis, C. intestinalis, Halocynthia papillosa and Microcosmus sabatieri at ambient concentrations of 2.10^7 cells.l^{-1} Monochrysis lutheri. Monochrysis lutheri is considerably smaller than Tetraselmis seucica; however, the size difference is not enough to explain the apparent differences with the present results. Different algal species, though, will not necessarily be digested and assimilated with the same efficacy, perhaps explaining this discrepancy. There is a decline (to 15%) in the absorption efficiency of Phallusia mammillata at a concentration of 2.10^8 cells.l^{-1} (Fiala-Médioni, 1979a) showing that the assimilation efficiency of this ascidian is also dependent upon the ambient suspension concentration. In bivalve molluscs, assimilation efficiency has been shown to be inversely related to suspension density (Stickney, 1964; Widdows and Bayne, 1971; Thompson and Bayne, 1974; Bayne et al., 1976; Winter, 1977, 1978; Widdows, 1978a; Griffiths and King, 1979). Allen (1962) pointed out that assimilation efficiency was not directly related to the suspension density, but to the ingestion rate. Foster-Smith (1975b) further qualified this view, suggesting that the important factor is the total amount of 'food' ingested over a given period. Griffiths and King (1979) have found that the assimilation efficiency of the ribbed mussel (Aulacomya ater)declines to zero at high cell concentrations. With regard to the results of other authors, this seems somewhat unlikely.

At 10^7 cells.l^{-1} the faeces of C. intestinalis are full (see Chapter 5) and the gut satiated. Further increases in suspension density would hence not increase the ingestion rate above that attained

at 10^7 cells.l^{-1}. It would, therefore, seem probable that the assimilation efficiency would decline no further with further increases in suspension density. This pattern has been suggested for lamellibranch bivalves by Winter (1977, 1978). The assimilation efficiency does not decrease, below the level found at 10^7 cells.l^{-1}, with the addition of 9 mg.l^{-1} Fuller's earth, when estimated by the cell count method. Similarly the assimilation efficiency, as estimated by the Conover ratio remains constant between 10^4 and 10^6 cells.l^{-1} with the presence of 9 mg.l^{-1} Fuller's earth. The reduced efficiency at 10^3 cells.l^{-1} is most likely due to inaccuracy in the method, there being a very low organic content in the food and the faeces at this concentration. It would thus appear that assimilation efficiency is a declining function of ingestion rate; the efficiency remaining constant (for any one particular food type) once the gut is satiated. Since little change was found in the gut residence times of satiated and unsatiated animals, the decline in assimilation efficiency must primarily be an effect of the volume of food in the gut. The effect of undigestible inorganic particulate material is, therefore, to reduce the efficiency of assimilation of ingested organic matter, when the organic suspension load is not enough to satiate the gut, and to reduce the quantity of organic material available for assimilation when the organic suspension load is sufficient for gut satiation. In the latter instance, though the assimilation efficiency is unchanged, the total amount of material assimilated is reduced.

The three methods used all have their merits and demerits. The cell count method is tedious and involves the sacrifice of the animals. In addition, very small quantities of food cord must be weighed. As the microscope slides, with their respective portions of food cord,

were allowed to equilibrate to the humidity and temperature of the balance room, and were all weighed during the same short time period, the precision of the weighing was high. Any inaccuracy involved would hence cancel out in the assimilation efficiency equation. The Conover ratio method has the advantages that animals are not sacrificed and that the quantitative analysis of faeces and ingested food is not necessary. The method can only be used with non-selective filter-feeders (Kiørboe et al., 1980; Kiørboe and Møhlenberg, 1981). Its use is, therefore, justified for ascidians. Forster and Gabbott (1971) have criticised this method on the grounds that some of the inorganic material may be assimilated. This criticism must be particularly valid for lamellibranch molluscs in which there is extensive intracellular digestion (Owen, 1966). Johannes and Satomi (1967) have suggested that additional inaccuracy may be caused by the release of unassimilated food, in the faeces, by dissolution into the water. Despite these drawbacks, Widdows and Bayne (1971) have shown that the Conover method gives a good estimate of the true efficiency. A further complication with this method is the presence of mucus in the faeces, causing an underestimate of the true assimilation efficiency. This factor was found to have a significant effect only at very low feeding levels, resulting in a reduction of the estimate from 90-100% to 72% (Fig. 35). The Maynard and Loosli method shares the criticisms of the Conover method, put forward by Forster and Gabbott and Johannes and Satomi (loc. cit.). The mucus content of the faeces is, however, of little consequence. The method involves neither the sacrifice of animals, nor the quantitative collection of faeces. A larger quantity of faeces are required, however, than with the Conover method, as both ash and protein weights are required. The method is also more time consuming, than the Conover method, due to the protein analyses.

Protein and total organic material are, in addition, subject to slightly different assimilation efficiencies (Pandian, 1967).

The finding here of a high assimilation efficiency for protein,and in the studies of Fiala-Medioni (1973, 1974, 1979a),seems to conflict with reports that ascidians have only very weak proteases (Yonge, 1925; Van Weel, 1940; Barrington, 1962; Goodbody, 1974). The answer may lie with intracellular digestion. True phagocytosis (as defined by the, now outmoded, criterion of the intake of visible particles) is absent in ascidians (Van Weel, 1940), but macromolecules may be directly absorbed without extracellular digestion, by a process of pinocytosis (Thorndyke, 1977; Burighel, 1979).

CHAPTER 7

"The influence of inorganic particulate suspensions on the growth and survival of the ascidians Ciona intestinalis L. and Ascidiella scabra Müller".

INTRODUCTION

Inorganic particulate suspension, above a certain threshold concentration, reduce the filtration rates of C. intestinalis and A. scabra, such that ingestion rates remain constant (Chapters 1, 2 and 5). The assimilation efficiency of any one particular food type is a function of total ingestion rate, not the quantity of that food type ingested (Chapter 6). On account of these phenomena and the fact that there is no particle selection (Chapters 3 and 5), it might be inferred that inorganic particulate suspensions would limit the intake of utilisable food by a similar process of dilution as described for bivalve molluscs by Foster-Smith (1975b) and Widdows et al., (1979). Inorganic particulate suspensions are, thus, likely to have an adverse effect on growth. At high enough concentrations, they may even be lethal to ascidians. The growth rates of C. intestinalis and A. scabra, at concentrations of Fuller's earth equivalent to particulate loads found naturally at various coastal sites, have been studied. In addition, the survival of C. intestinalis at concentrations of Fuller's earth equivalent to those of natural particles that might be induced by dredging, marine mining or marine construction operations have been investigated.

MATERIAL AND METHODS

Growth of A. scabra

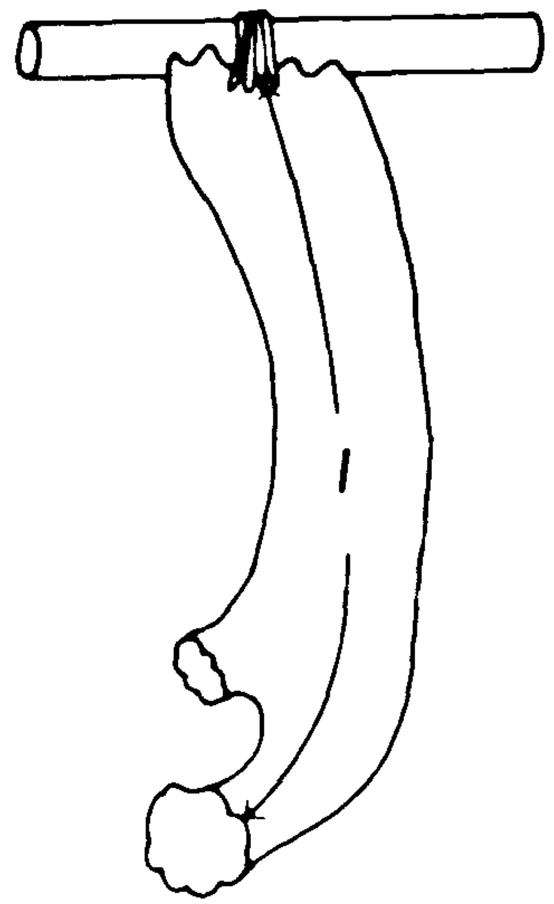
Small specimens (0,5-1,5cm length), still attached to Fucus fronds, were placed in 3-litre jars, 10 animals to a jar. Fuller's earth and Dunaliella salina suspensions were added twice daily. Mean concentrations of Fuller's earth were estimated with an absorptiometer (see Chapter 1). All jars received the same quantity of algal cells. The animals were removed from the tanks every fortnight and the parameters length (l), height (h) and breadth (b) (as illustrated in Fig.36, b and c) measured with vernier calipers. The product of these three measurements was used as a measure of volume (V). Mean volumes ($\bar{V}$) for the animals of each jar were plotted as a parameter of size.

Growth of C. intestinalis

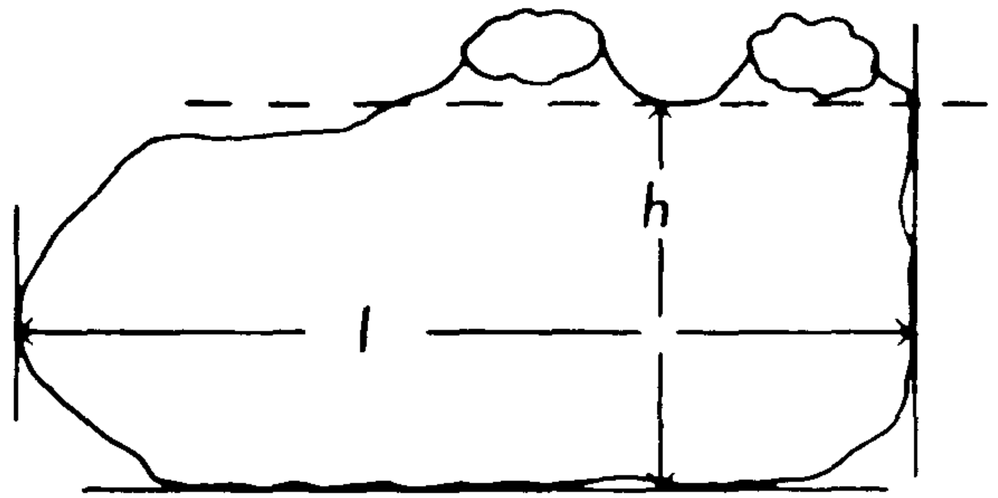
A parameter of size is obtained with more difficulty for C. intestinalis because it is more contractile (Millar, 1952, 1971a). Millar (1952) overcame this problem by narcotizing animals before once-off measurement. Weekly narcotization, however, was found to significantly reduce the growth rate of C. intestinalis. The best method of recording growth was found when using equipment designed to keep very high concentrations of Fuller's earth in suspension (described below). The experimental tanks being so thin, the animals were clearly visible even at 170 $mg.l^{-1}$ suspension load and they could be measured from the outside of the tank without disturbing them. For this purpose a calibrated length of flexible wire was used and the length (l) from the base to the tip of the oral siphon measured, taking into account any curvature of the body (Fig. 36a).

FIGURE 36 Parameters of size measured for a. C. intestinalis, b. and c. A. scabra.

a. and b. are lateral views
c. is a ventral view.

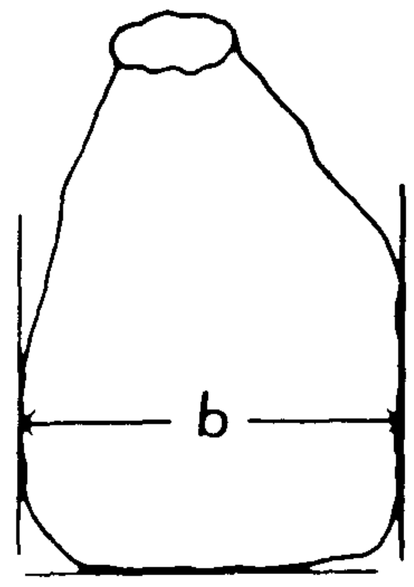


a.



b.

C. intestinalis



A. scabra

c.

Survival of C. intestinalis

The problem of maintaining high concentrations of inorganic particles suspended for long periods of time, without such vigorous water movement that experimental animals are unable to function properly, has bedevilled investigators (Moore, 1977). One of the major problems comprises the design of the floor of the experimental vessel. In these experiments, thin perspex tanks were constructed (dimensions 25 x 21 x 2cm). Air was bubbled into the tanks at one end to provide circulation of the water within the tank. About 100g of Fuller's earth was added to each tank, and the tanks left for a week. The Fuller's earth settled on to parts of the tank floor in inverse proportion to localised water movements. Thus, the sedimented Fuller's earth formed a 'false-floor' in such a way as to eliminate any 'dead-space' (see Moore, loc. cit.). Once such a 'false-floor' had been formed, it was found that particles could be kept in suspension for long periods, with minimal water movement. Thin tanks were used such that animals could be seen even at the highest concentrations of suspended particles investigated.

Medium sized C. intestinalis (2-6cm length) were attached by their bases, with thread, to glass rods (10 to a rod). A glass rod was then suspended in each thin tank. Suspensions of Fuller's earth in seawater, or seawater alone (control), were pumped into the tanks from 5l round-bottomed flasks (see Chapter 5). The animals were fed with algal culture (Tetraselmis seucica) twice daily as in the growth experiments. The number of deaths and the concentration of the particulate suspensions were measured daily.

All experiments in this study were carried out at 15 $\pm$ 2^{o}C.

RESULTS

Survival of C. intestinalis

C. intestinalis cannot survive for long periods in high concentrations of Fuller's earth. At concentrations between 602 and 2424 $mg.l^{-1}$ the survival curves are very similar; 100% mortality occurring, in each case, after about 3 weeks and 50% mortality occurring between 12 and 15,5 days (Fig. 37).

Growth

Both C. intestinalis and A. scabra can survive in lower concentrations of Fuller's earth, when ample utilisable organic food is available (Figs. 38 and 39). Normal growth is however impaired. Good growth was recorded for both species in control tanks. C. intestinalis grew somewhat faster than A. scabra, increasing its length by 30% over 42 days compared with a 40% volume increase of A. scabra in 84 days. Concentrations of 25-170 $mg.l^{-1}$ Fuller's earth effectively arrested the growth of C. intestinalis. The growth rate of A. scabra, however, was progressively reduced by increasing concentration of Fuller's earth, but only completely arrested at 120 $mg.l^{-1}$. A portion of the assimilated energy of C. intestinalis is used to produce attachment villi. An experiment involving the narcotization of animals prior to measurement was abandoned after 2 weeks. The strength of the attachment and hence growth of the villi was estimated, however, at the end of this period, the animals having been unattached at the beginning of the experiment. The number of animals attached and the strength of this attachment was reduced in increasing suspension loads of Fuller's earth, the animals being incapable of re-attachment, within 2 weeks, at 120 $mg.l^{-1}$ (Table 17).

FIGURE 37 Survival of C. intestinalis exposed to high concentrations of Fuller's earth.

——— = control

○·····○ = 602 ± 142 mg.l^{-1}

●- - - -● = 1295 ± 632 mg.l^{-1}

△—·—·—△ = 2424 ± 859 mg.l^{-1}

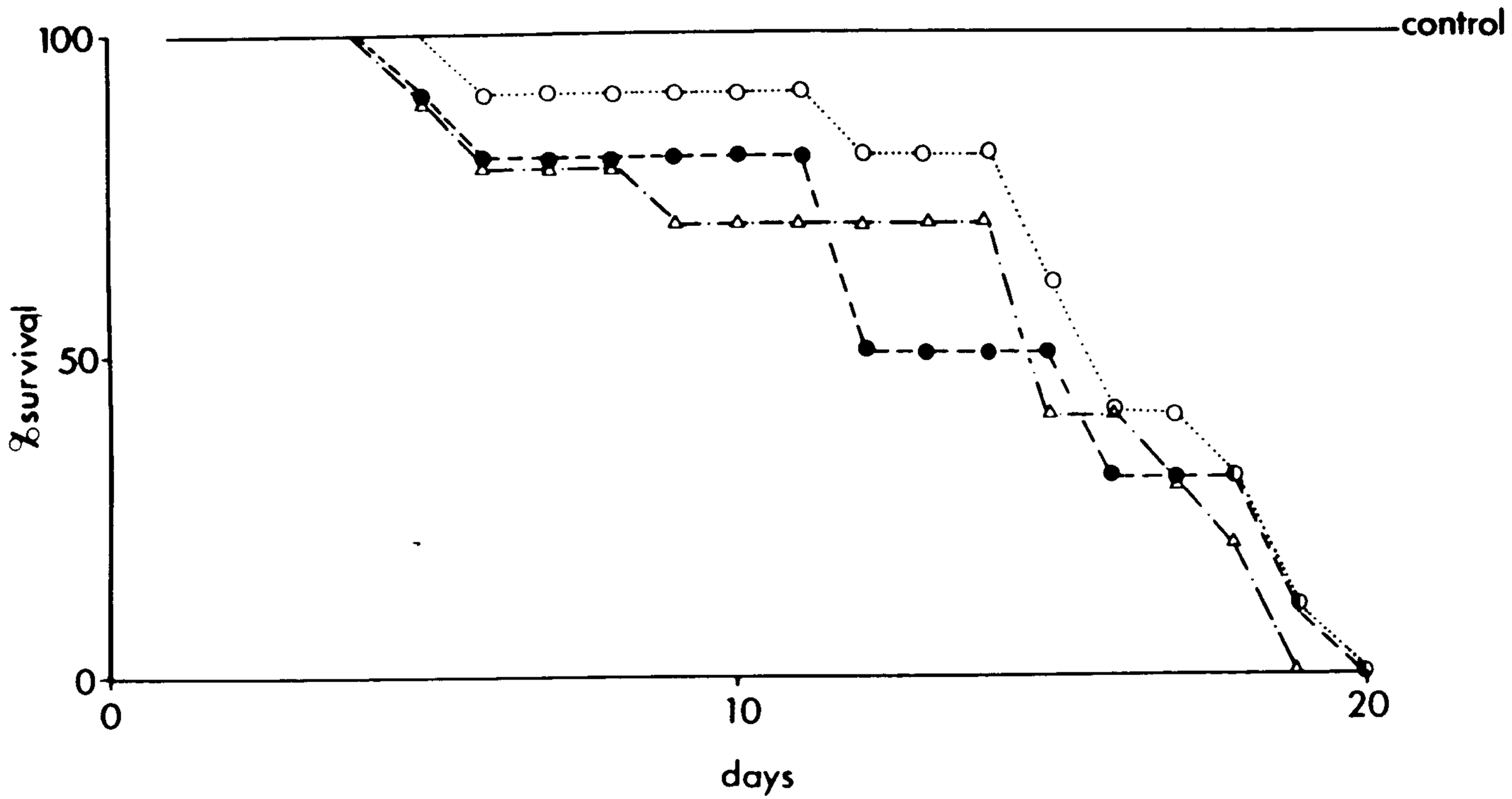

control
100
50
0
% survival
0
10
20
days

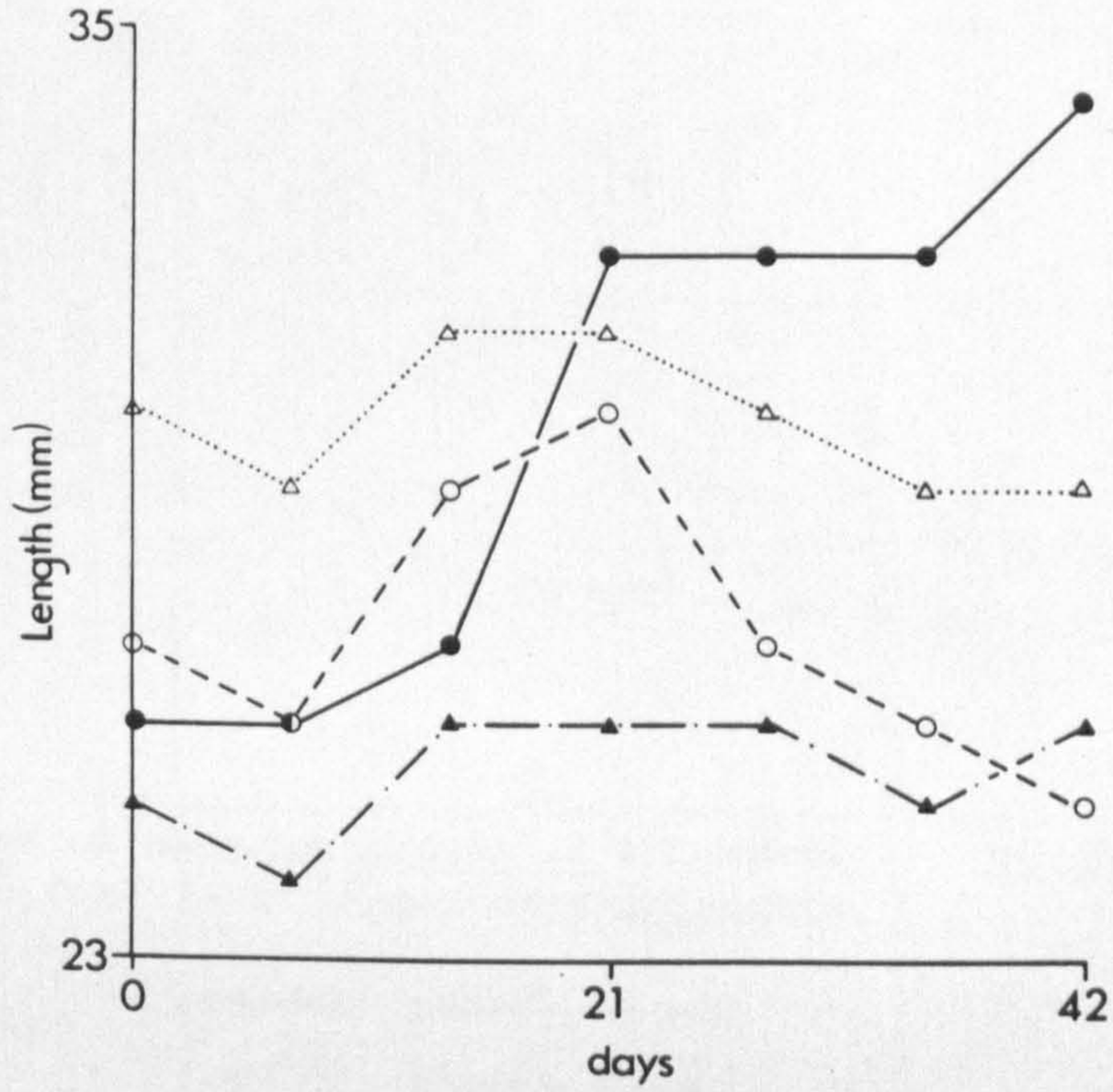
FIGURE 38 Growth of C. intestinalis exposed to various concentrations ($mg.l^{-1}$) of Fuller's earth.

●———● = control

○- - - -○ = 25 $mg.l^{-1}$

▲—·—·▲ = 80 $mg.l^{-1}$

△·····△ = 170 $mg.l^{-1}$


35
23
Length (mm)
0
21
42
days

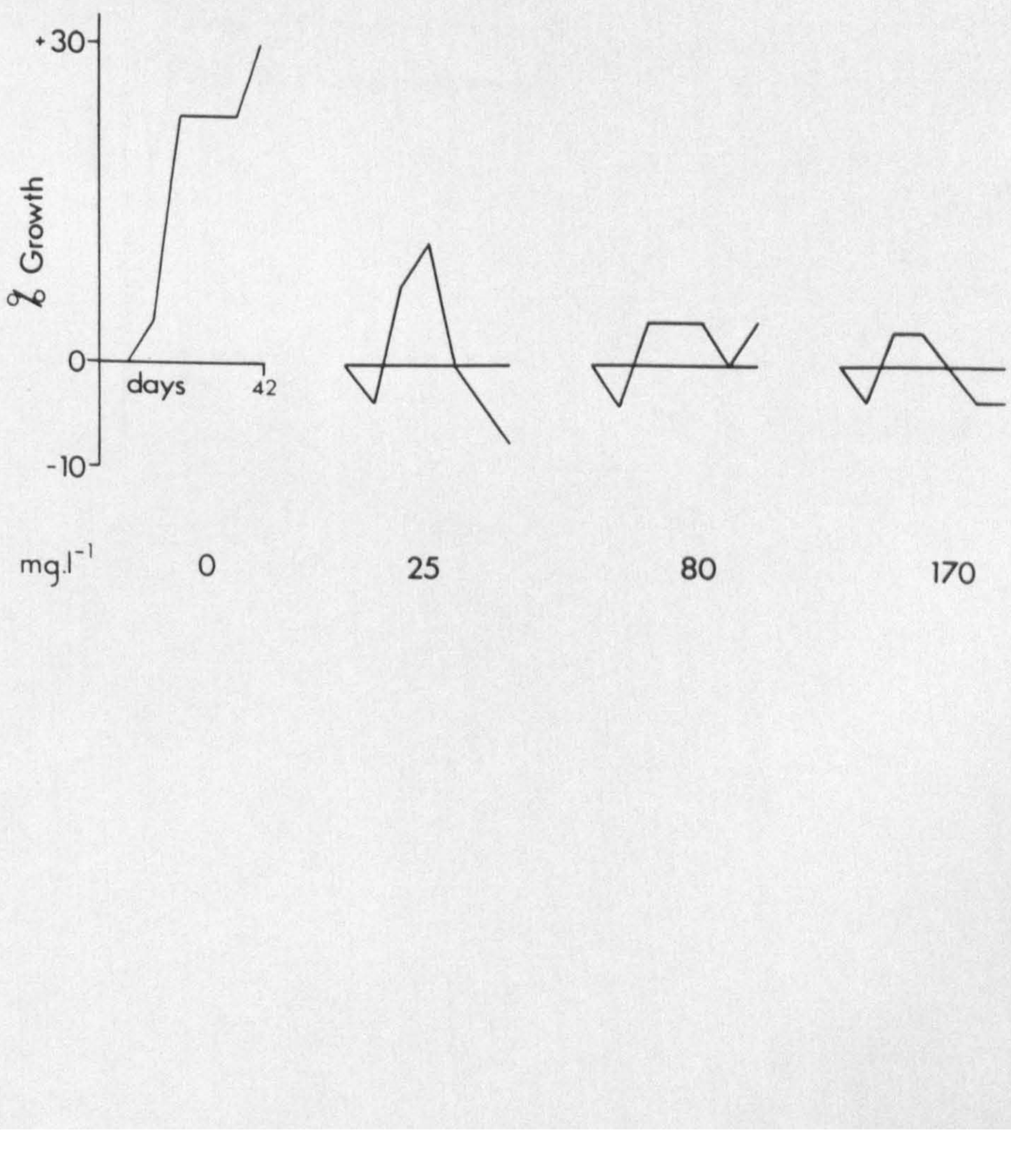

+30
0
-10
% Growth
days
42
mg.l-1
0
25
80
170

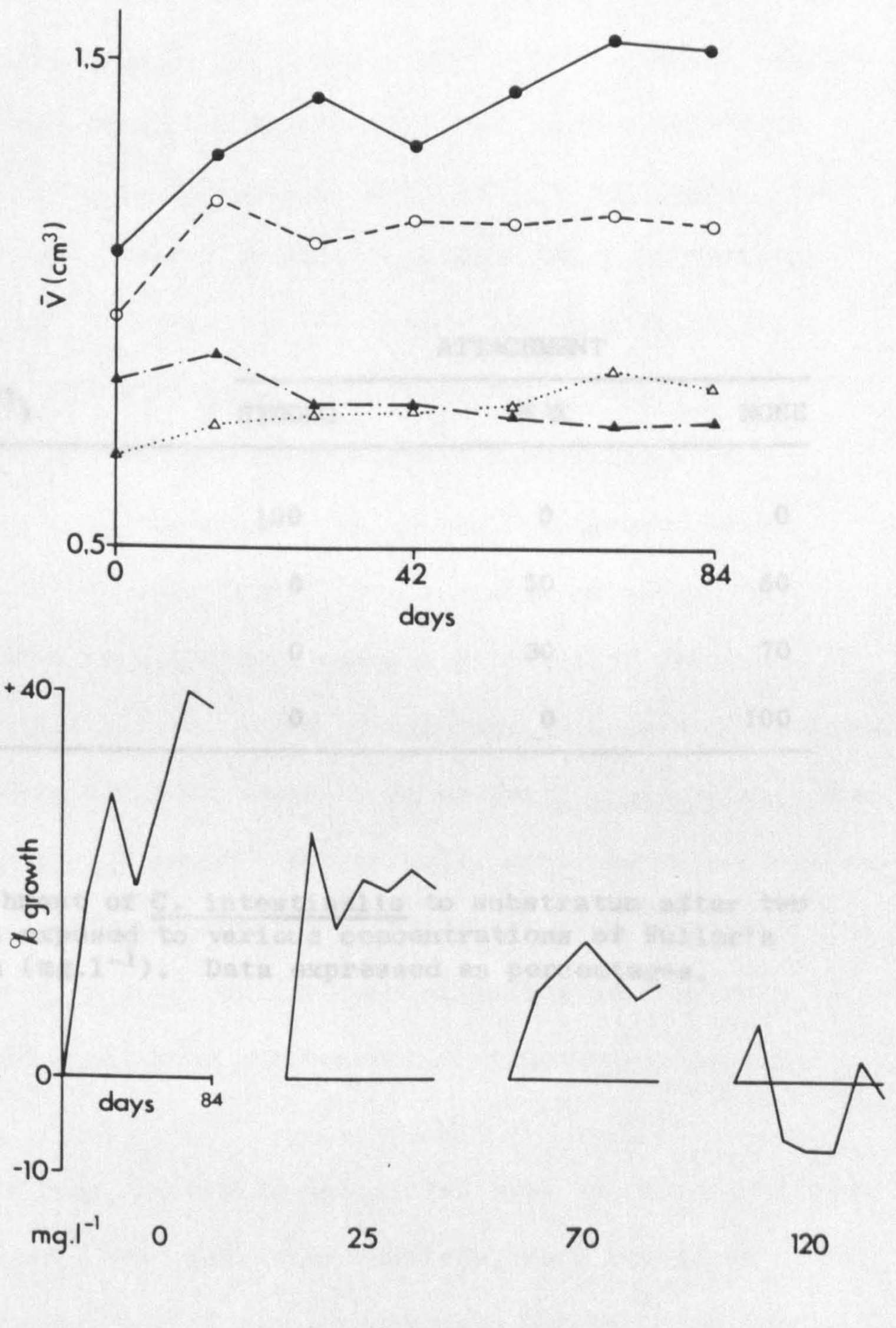
FIGURE 39 Growth of A. scabra exposed to various concentrations ($mg.l^{-1}$) of Fuller's earth

●——●= control

○- - - -○= 25 $mg.l^{-1}$

△·····△= 70 $mg.l^{-1}$

▲—·—·—·▲= 120 $mg.l^{-1}$


1,5
0,5
$\bar{V}$ (cm^3)
0
42
84
days
+40
% growth
0
-10
days
84
mg.l^{-1}
0
25
70
120

CONCENTRATION ($mg.l^{-1}$)	ATTACHMENT		
	STRONG	WEAK	NONE
0	100	0	0
25	0	50	50
70	0	30	70
120	0	0	100

TABLE 17 Attachment of C. intestinalis to substratum after two weeks exposed to various concentrations of Fuller's earth ($mg.l^{-1}$). Data expressed as percentages.

DISCUSSION

The growth of filter-feeding animals in temperate waters has been correlated classically to seasonal temperature fluctuations. Ascidians are no exception (Millar, 1952, 1953, 1954, 1971a; Jang, 1979). Millar (1953) pointed out that available food is probably also a factor of prime importance influencing the growth rate of C. intestinalis. The true significance of this factor to filter-feeders has only recently been realised (Thompson and Bayne, 1974; Winter and Langton, 1976; Winter, 1976; Murken, 1976; Incze et al., 1980; Vahl, 1980). The quality of the seston may in some areas (if not universally) be a more important factor than temperature in controlling growth rate (Bayne and Widdows, 1978; Vahl, 1980). The addition of inorganic material to the water column, through natural (storms) or unnatural (dredging, mining, etc.) causes, would be expected to have a detrimental effect on growth. This has been found to be so for C. intestinalis and A. scabra. C. intestinalis is more susceptible, very low concentrations of inorganic material arresting growth. The growth rate is greatly reduced, however, in A. scabra, by low concentrations of inorganic material. Under such conditions the production of gametes might be impaired.

Higher concentrations, as may be associated with dredging or mining and natural processes in some estuarine habitats, were lethal to C. intestinalis. The pharyngeal mechanism became blocked with inorganic material only shortly before death, suggesting that the animals were capable of keeping their feeding mechanisms functional for a substantial length of time. The major cause of death would appear to be the animals' incapability of assimilating enough energy to satisfy metabolic maintenance costs.

Mortality and reduced growth rate have been associated with inorganic particulate suspensions in bivalve molluscs (Loosanoff, 1962; Turner, 1971; Chang and Chin, 1978; Incze et al., 1980) and the slipper limpet Crepidula fornicata (Johnson, 1972). Very low concentrations of inorganic material,however, reportedly accelerate the growth rate of bivalve molluscs (Davis, 1960; Millar and Scott, 1968; Davis and Hidu, 1969; Winter, 1976; Murken, 1976). Several mechanisms have been proposed to account for this. Inorganic particles have been regarded as chelating or adsorbing toxins in the water and, hence, enhancing growth (Davis, 1960; Millar and Scott, 1968; Davis and Hidu, 1969). Murken (1976) suggested that inorganic particles might have a grinding effect on ingested algae, thus enhancing their digestion. Utilisation of some of the organic matter originating from silt has been demonstrated by Kiørboe et al., (1980) in the mussel Mytilis edulis. This possibility was eliminated in the works of Winter and Murken by stripping organic matter from silt prior to experimentation. It is possible, however, that the particles may have adsorbed organic material present in the water, or developed a bacterial flora, during the experiments. The presence of lysozyme in the gut of M. edulis and other bivalves, has been demonstrated by McHenery et al., (1979) and a bacterial flora on inorganic particles might be expected to provide a scource of nourishment.

Such an enhancement of growth was not found with C. intestinalis and A. scabra, but it is possible that the suspended inorganic particulate concentrations were too high. The assimilation efficiency of C. intestinalis is a function of ingestion rate (Chapter 6) and it is unlikely that inorganic particulates could enhance growth. Selection of particles in the stomach complicates the issue in bivalve molluscs. It is possible that algal cells and inorganic particles are separated in the stomach,

the former being passed to the digestive gland, the latter passing straight through the gut (Foster-Smith, 1975b). If this is so, the assimilation efficiency of algae ingested and channelled to the digestive gland will not be altered by the presence of small quantities of inorganic particles. If the gut is satiated however, the quantity of cells ingested might be reduced by a process of 'dilution' (Foster-Smith, 1975b; Widdows et al., 1979). It is by such a process of 'dilution' that fewer algal cells are ingested by ascidians, in the presence of suspended inorganic particulates.

"Integrated analysis of the effects of inorganic particulate suspensions on the feeding of ascidians, with a note on the bioenergetics of C. intestinalis".

i. The effects of inorganic particulate suspensions on the feeding of ascidians

The work presented in this thesis involves the primary effects of inorganic particulate suspensions (i.e. the effects of their physical presence within the water column). Secondary effects (e.g. changes in water chemistry, light regimes, etc.), associated with the suspensions, are not considered. These primary effects are summarised in a flow diagram (Fig. 40). The inorganic particulate suspensions act primarily by increasing the seston concentration, this being a factor of great importance to filter-feeding animals. The naturally occurring seston will, of course, contain a proportion of inorganic material. For ease of explanation, however, it is convenient to include this fraction with the inorganic particulate suspension added to the water. It is now possible to define two extremes of reaction by ascidians. The first is initiated when the total seston concentration (including the added inorganic particulates) is not sufficiently great that animals satiate their guts (solid arrows in Fig. 40). Under such conditions, the additional inorganic particulate concentration will have little effect on absolute pumping rates or squirting rates and, hence, no effect on filtration rate. Since ingestion rate is a function of filtration rate and seston concentration, the total ingestion rate will be increased. But the concentration of organic matter within the water remains unchanged, as does its rate of ingestion. The efficiency with which this organic matter is assimilated however, is dependent upon total ingestion rate and, hence, is reduced. The overall effect is to reduce the quantity of organic matter assimilated and, hence, the energy available for growth and maintenance.

The second reaction is initiated when the organic matter naturally present in the water is at a sufficiently high concentration that animals satiate their guts with it alone (broken line in Fig. 40).

FIGURE 40 A summary of the effects of inorganic particulate suspensions on the feeding of ascidians.

Pabs = absolute pumping rate

S = rate of squirting

F = filtration rate

I_t = total ingestion rate

Io = organic ingestion rate

As% = assimilation efficiency

AsO = amount of organic matter assimilated.

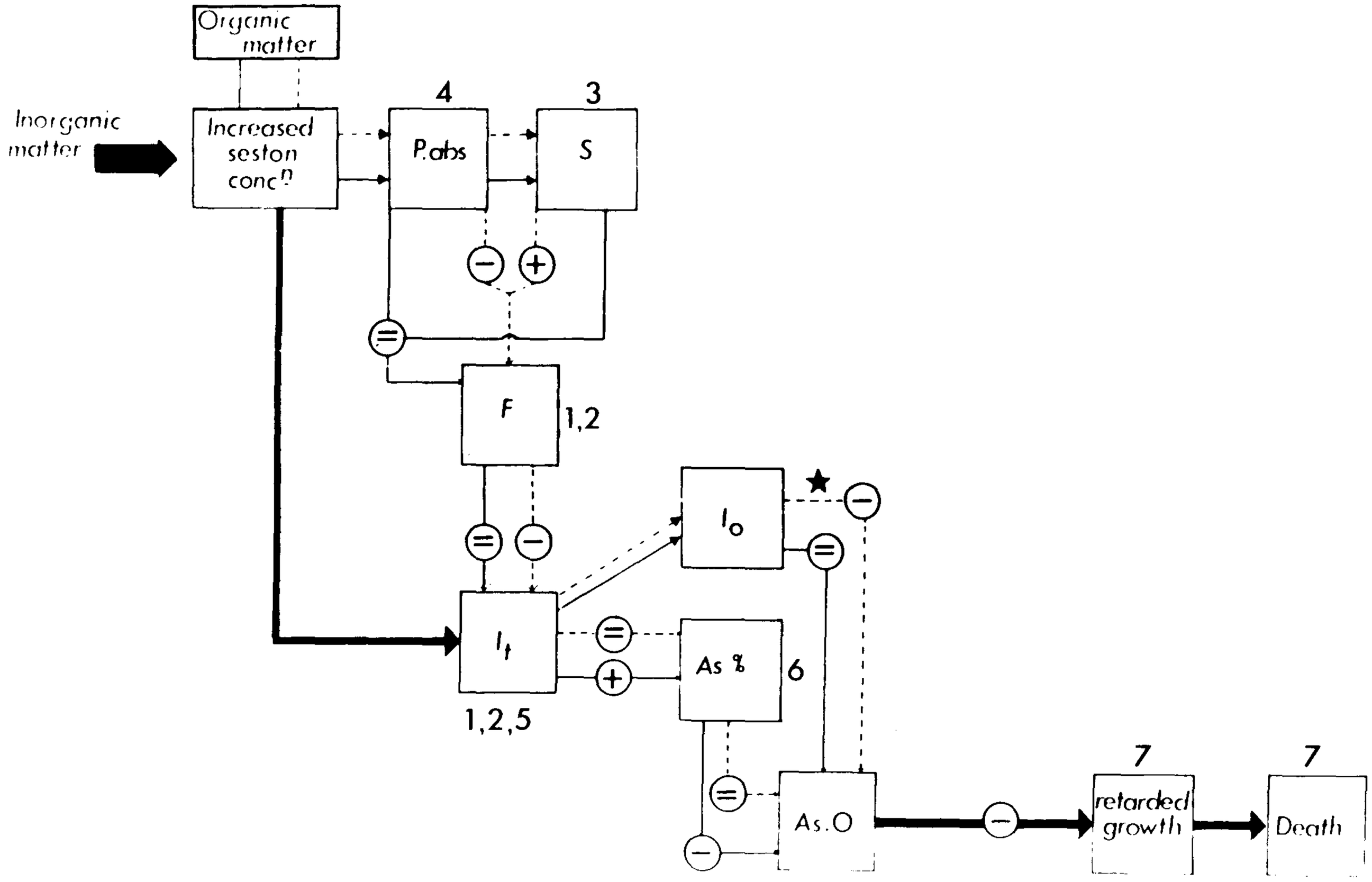
\+ = increases function

— = decreases function

= = no change in function

★ = 'dilution' effect

Numerals refer to chapters concerning each particular step.


Organic matter
Inorganic matter
Increased seston conc^{n}
4
P.abs
3
S
F
1,2
I_o
I_f
1,2,5
As %
6
As.O
7
retarded growth
7
Death

Under such conditions, the addition of an inorganic particulate suspension will cause a reduction in absolute pumping rate and an increase in the rate of squirting. These two factors serve to reduce the filtration rate, such that the total ingestion rate remains constant. The rate of ingestion of organic material will be reduced, however, due to its effective dilution in the seston (see Chapter 7). The efficiency with which this organic material will be assimilated is unaltered, as the total ingestion rate remains constant. The overall effect is, as above, a reduction in the quantity of organic matter assimilated. A range of intermediate reactions is possible, when the organic matter alone is not concentrated enough for the animals to be able to satiate their guts; but the total seston concentration is. Under such conditions there will be a reduced filtration rate and reduced assimilation efficiency. The total ingestion rate will be increased, but the organic ingestion rate decreased. The nett effect will be the same as that involved in the two extreme reactions detailed above.

ii. A note on the bioenergetics of C. intestinalis

Bioenergetics have not been considered in this study. Some of the results, however, can be used to give an indication of the energy status of C. intestinalis filtering different concentrations of Tetraselmis seucica. Fisher (1977) has proposed a simple index of energy status, the "relative maintenance cost". This index is based upon the balance between energy equivalent losses through oxygen consumption and energy gains through feeding. As the gut becomes satiated only at the highest concentrations of algal cells used, it will be assumed that the filtration rate and the ratio of filtration rate to oxygen consumption ($l.mlO_2^{-1}$) remain constant. The amount of organic material ingested will be the product of filtration rate and the concentration of organic material

suspended in the water column. The relative maintenance cost = $\frac{\text{energy losses}}{\text{energy gains}}$. Values greater than unity, therefore, indicate that the maintenance requirement cannot be met by the energy absorbed, whereas values less than unity represent relatively lower maintenance costs, with energy available for growth. Energy losses due to oxygen consumption are:-

$$\frac{F.4,75}{\beta}$$

where F is the filtration rate ($l.h^{-1}$), β is the ratio of filtration rate to oxygen consumption ($l.mlO_2^{-1}$) and 4,75 is the oxycalorific conversion constant ($cals.mlO_2^{-1}$). Energy gains are:-

$$F.POM.k.As$$

where POM is the concentration of suspended organic matter ($mg.l^{-1}$), k is the conversion factor from mg suspended organic matter to energetic equivalents ($cals.mg^{-1}$) and As is the assimilation efficiency. Thus

relative maintenance cost =

$$\frac{F.4,75}{\beta.F.POM.k.As}$$

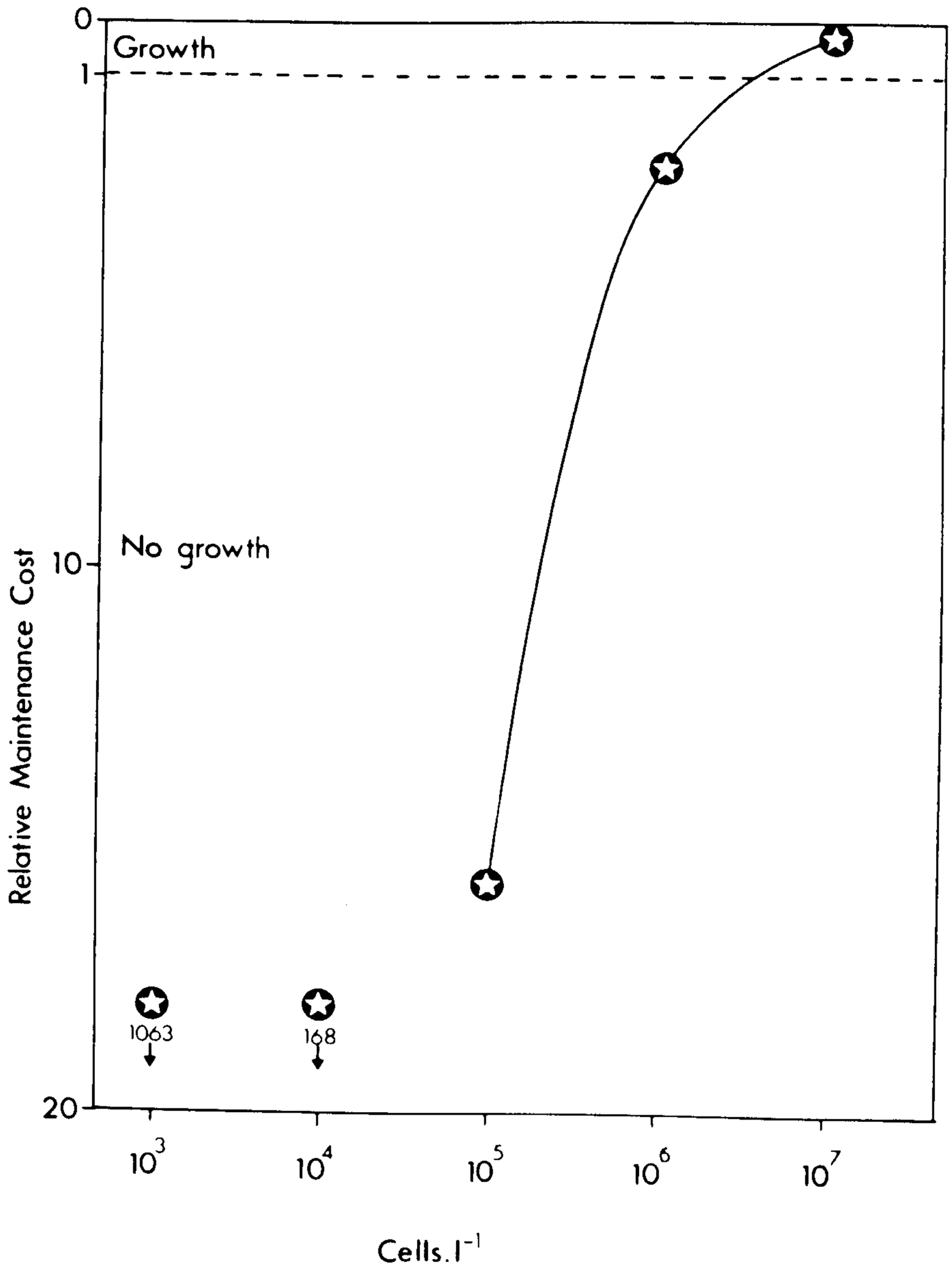
which reduces to:-

$$\frac{4,75}{\beta.POM.k.As}$$

This final equation is identical to that given by Bayne and Widdows (1978). Substituting the parameters of this equation for dimensions, we get:-

$$\frac{Cals.mlO_2^{-1}}{l.mlO_2^{-1}.mg.l^{-1}.cals.mg^{-1}}$$

FIGURE 41 The relative maintenance costs of C. intestinalis feeding on T. seucica at various concentrations (cells l^{-1}).


0
Growth
1
No growth
10
20
Relative Maintenance Cost
1063
168
10^3
10^4
10^5
10^6
10^7
Cells.l^{-1}

This equation completely cancels itself out, and the relative maintenance cost, therefore, is dimensionless. The weight of 10^6 Tetraselmis seucica cells is 0,066 mg. and its energy content (k) is 5,6 cals. per mg. (dry weight), (Widdows and Bayne, 1971). A value of 13,0 has been given for β by Jórgensen (1955). Using these values and values of As given in Chapter 6, relative maintenance costs have been calculated for various concentrations of T. seucica. It would appear, from these results, that the metabolic maintenance costs are only met at concentrations well in excess of 10^6 cells.l^{-1}. C. intestinalis would, hence, be expected to be very sensitive to any decrease in the amount of energy assimilated caused by low concentrations of inorganic particulate suspensions.

As the reduced pumping rate associated with high suspended particulate concentrations is probably due to the partial blocking of the mucus filter, rather than a decrease in the rate of beating of the stigmatal cilia, and there is an increase in the squirting activity, β is unlikely to remain constant. Values of the relative maintenance cost cannot, therefore, be calculated, in this study, for conditions when the gut becomes satiated.

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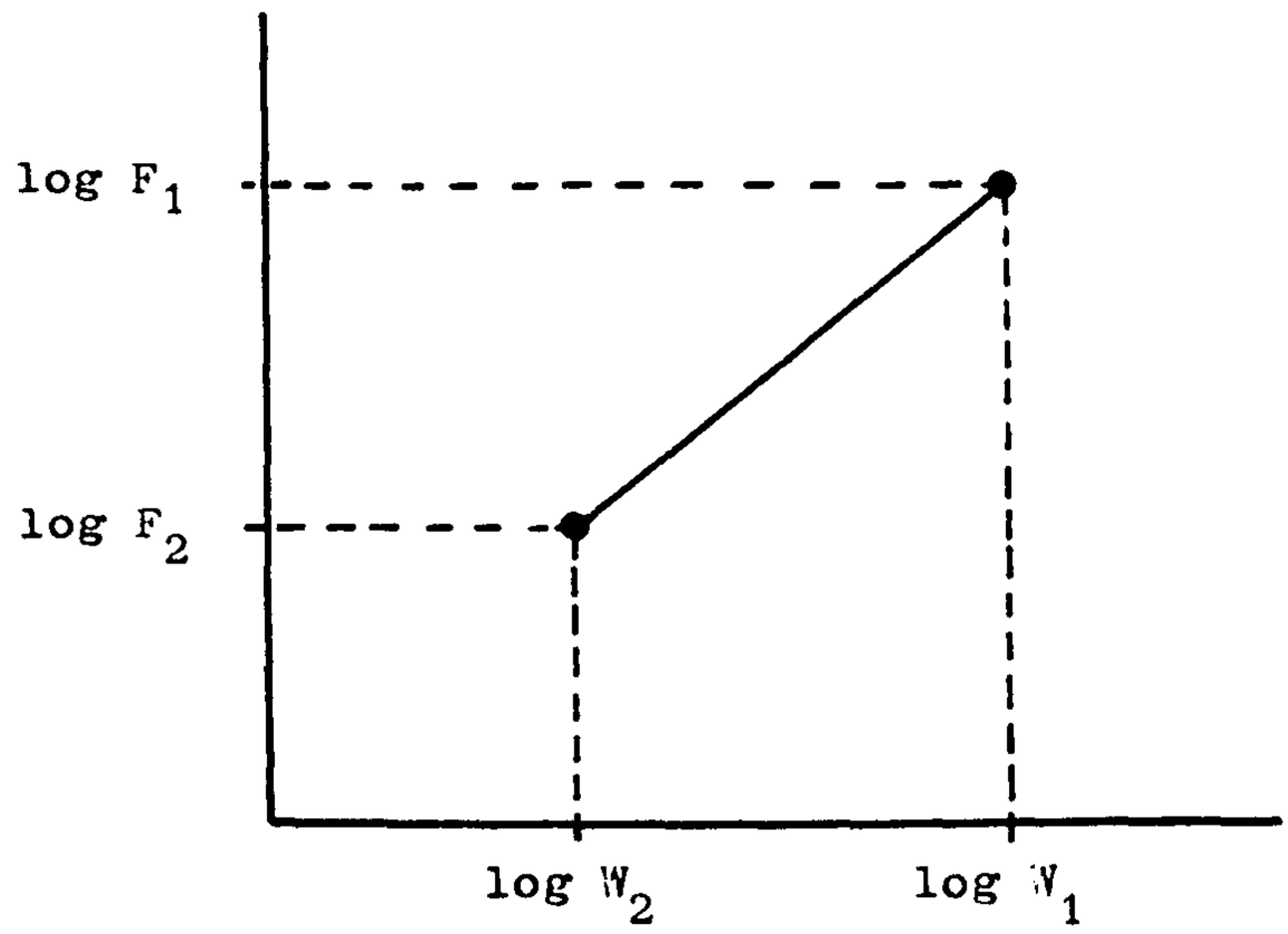
APPENDIX



Consider two data points; F_1, W_1 and F_2, W_2 when log transformed.

The gradient a_f between them is:

$$\frac{\log F_1 - \log F_2}{\log W_1 - \log W_2} \quad \ldots\ldots \quad 1$$

equation 1 can be rearranged to:

$$a_f = \frac{\log (F_1/F_2)}{\log (W_1/W_2)} \quad \ldots\ldots \quad 2$$

hence:

$$\log \frac{F_1}{F_2} = \log \frac{W_1}{W_2}^{a_f} \quad \ldots\ldots \quad 3$$

or

$$\frac{F_1}{F_2} = \frac{W_1}{W_2}^{a_f} \quad \ldots\ldots \quad 4$$