CONTRIBUTIONS TO THE ECOLOGY
OF THE
BENTHIC MACROFAUNA
OF THE
BOT RIVER ESTUARY

by

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Thesis submitted in fulfilment of the requirements for the degree of Master of Science in the Faculty of Science, University of Cape Town.

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To my parents

M.M. De Decker

and

the late "Doc" A.H.B. De Decker

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To work and yet not pay life's price for working:
to live; yet not renounce the work of creation.
Could it ever be done?

Herman Hesse

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GENERAL INTRODUCTION

This thesis forms part of a multi-disciplinary study of the Bot River estuary, situated between Kleinmond and Hawston on the southwest coast of the Cape Province, and falls within the framework of the investigation of all Cape estuaries, initiated by SANCOR and co-ordinated by the Estuarine and Coastal Research Unit of the CSIR. The motivation for the research program on the Bot River estuary, in particular, was the need to obtain detailed knowledge of its dynamics in order to be able to address its unique management problems.

The estuary is normally separated from the sea by a sandbar across its mouth, but unlike many other South African estuaries, this is rarely breached naturally, due to the overflow of water at high levels to the neighbouring Kleinmond estuary. As a result, recruitment of fish from the sea is prevented, while increased freshwater input may cause flooding of the adjacent properties and a reduction in salinity to levels which are fatal to fish and promote the abundant growth of "weeds" (the macrophytes Ruppia maritima and Potamogeton pectinatus). These are adverse effects for local landowners, fishermen and recreational users of the estuary. They can be temporarily reversed, however, by artificially breaching the sandbar that blocks the mouth, but this has an immmediate catastrophic ecological impact. A massive drop in water levels occurs, exposing most of the Ruppia maritima beds, which then become desiccated, die and rot, while the salinity of the remaining water approaches that of seawater. It is clear that any effective management plan for the estuary must take into consideration both the importance of human utilisation and the ecological implications of mouth-breaching. This can only be achieved if it is based on a sound knowledge of the system.

This thesis is a contribution towards the understanding of the benthic macrofauna of the Bot River estuary. Previous knowledge of the benthos was confined to a single, limited survey conducted as a practical exercise by undergraduate Zoology students at the University of Cape Town, and is referenced in Chapter One. The dissertation comprises a series of independent papers, each containing the literature, details of the study site and methods relevant to its particular aims.

The first chapter gives a general description of the species composition, abundance, biomass and distribution of the benthos. Tentative conclusions are drawn concerning the importance of environmental factors in determining the structure of benthic communities in the estuary. The results of this work suggested the possible significance of interactions between wind, currents, turbidity and colonisation by Ruppia maritima in influencing the distribution of the benthic macrofauna. A detailed survey encompassing all these factors was planned, but before it could be launched, the mouth of the estuary was breached (29 June 1983) and the survey literally stranded. An investigation of the interactions became impossible, since the currents followed a tidal regime and most of the macrophytes were exposed.

On the other hand, the breaching provided an opportunity to study the effects of such a calamitous event on the benthos, and to monitor its recovery. This investigation is reported in Chapter Two.

When, some months later, the estuary had returned to its normal, non-tidal state, colonised by mature *Ruppia*, the interaction between the sediment distribution in the estuary and the species composition of invertebrate communities could be studied. The identification of the major sedimentary factors structuring the benthic macrofauna is discussed in Chapter Three, which also serves as an illustration of the application of multivariate statistical analysis to studies in estuarine ecology.

In an attempt to quantify the energy-flow through the consumer community, attention shifted to individual species. Two species, the bivalve *Arcuatula capensis* and the isopod *Exosphaeroma hylecoetes*, which together contributed more than 85% to the total biomass of the benthos, were selected. Four digestive enzymes of these species were assayed, with the aim of relating their activities to the natural resources available to consumers in the estuary. This technique has been used previously to determine the carbon budgets of invertebrates, but calculation of carbon yield from enzyme activities has only very recently been shown to be inappropriate and therefore invalid. Chapter Four discusses these problems with reference to four digestive enzymes of the crystalline style of *Arcuatula capensis*, viz, three carbohydrases and a lytic enzyme.

The determination of enzyme activities for the isopod *Exosphaeroma hylecoetes* was rewarded, however, by the first experimental demonstration of the existence of a lytic enzyme in a crustacean. This is described in Chapter Five.

Food resources of the Bot River estuary have a further important role to play in the nutritional ecology of its consumer population. Many fish species are known to migrate between the sea and open estuaries, presumably benefitting from their greater food availability. This presumption has never been tested, however. The closure of the Bot River estuary for a period of four years (1977 to 1981), afforded an opportunity of testing this assumption, by comparing the physiological condition of the resident (trapped) southern mullet, *Liza richardsoni*, with that of conspecifics in the sea. This comparison is made in Chapter Six.

Finally, in the General Conclusions, hypotheses generated by this thesis, concerning the important environmental and biological interactions in the system, are drawn together into a single testable framework which can serve as a springboard for further research into the ecology of the Bot River estuary.

NOTE

1. As a result of the independent nature of the chapters, some overlap of material has been unavoidable, particularly in the descriptions of the study site and methods. However, repetition has been limited as far as possible.

2. The two published chapters have dual authorship, but the contributions of the co-authors are restricted either to work falling outside the scope of this thesis (Bally, Chapter One (meiofauna)), or to assistance of a supervisory nature (Bennett, Chapter Six). The Supporting Paper, of which I am a co-author, has been included to facilitate understanding of the management problems of the Bot River estuary.

CHAPTER 1

The benthic macrofauna of the Bot River estuary, South Africa, with a note on its meiofauna

by

H.P. De Decker and R. Bally

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THE BENTHIC MACROFAUNA OF THE BOT RIVER ESTUARY, SOUTH AFRICA, WITH A NOTE ON ITS MEIOFAUNA

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SUMMARY

The species composition, abundance, biomass and distribution of the benthic macrofauna of the Bot River estuary are discussed. In general, the fauna is impoverished, comprising mainly amphipods, isopods, polychaetes and molluscs. Average total biomass figures are 8,29 g dry mass m^{-2} for December 1982 and 4,32 g dry mass m^{-2} for May 1983. The tanaid, *Apseudes digitalis*, was the most abundant species, while the bivalve, *Arcuatula capensis*, had the highest biomass. Low diversities and abundances are attributed to the lack of contact with the sea. The estuary can be divided roughly into four major zones, based on the distribution and abundance of the benthic macrofauna. Faunistically, the zones differ with respect to the characteristic and numerically dominant species. Highest total biomass figures (91,63 g dry mass m^{-2}) are associated with the *Ruppia* beds, whereas the lowest figures were usually found in the deeper areas.

The meiofauna of the system is noticeably impoverished, in terms of both numbers and diversity. The main groups represented are the platyhelminths, nematodes and oligochaetes, with a few chironomids, polychaetes and juvenile forms of macrofaunal amphipods. Densities are low (0,02 to 1,82 cm^{-3}·sediment) and the animals are relatively large for meiofaunal organisms. The abundance and diversity of these groups are closely related to the composition of the sediment, with the richest sediments being those with the lowest mud fractions, close to the sea.

INTRODUCTION

Detailed work has been done over more than three decades on the ecology of selected estuaries in the Cape (Day 1951, 1967; Scott *et al.* 1952). However, no coordinated effort to investigate all 167 estuaries between the Orange and Kei Rivers, with the specific aim of formulating a cohesive management policy, had been attempted until 1979, when the Estuarine and Coastal Research Unit (E.C.R.U.) was established (Heydorn & Tinley 1980). As part of the project, intensive investigations of selected estuaries, usually with particular management problems, were begun. The Bot River estuary is an example of a system with unique management problems (Koop 1982, Branch *et al.* 1985), which motivated an intensive interdisciplinary research programme, starting in 1980.

Several studies are in progress, or have been completed (see Koop 1982 for a review of available information) and are reported on in this volume. Work on the benthic macrofauna, however, has been limited to part of a general ecological survey completed in 1979 by Koop *et al.* (1983). The present study comprises two general surveys of the Bot River estuary to determine the species composition, abundance, biomass and distribution of its benthic macrofauna. A brief report on the meiofauna of the estuary is included.

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STUDY SITE

The Bot River estuary (34°21′S 19°07′E) lies on the south-west coast of South Africa, near Kleinmond (Fig. 1). Detailed descriptions of the estuary may be found in Koop (1982) and Koop *et al.* (1983) and other papers in this issue. Only details pertinent to this study will be given here. The estuary is usually separated from the sea by a sandbar, but at high water-levels (due to fresh water input) a natural overflow channel opens to the sea via the swamps at Kleinmond. The water-releasing effect of this channel ensures that the sandbar is seldom breached naturally and the estuary is therefore 'blind' (Day 1951). Sea-water enters only after an artificial breaching of the mouth, approximately once every three years. The lagoon is 7 km long, 2 km wide at its widest point and has an area of about 6–13 km^2. The basin is shallow, with a maximum depth below mean sea-level (MSL) of −2.5 m. Water-levels fluctuate between MSL and a maximum of +2.7 m above MSL, depending on fresh-water input, evaporation and time since the last mouth breaching. Salinities range from 40‰ to as low as 5‰ (Koop 1982), but varied only from 21‰ to 23‰ between the surveys. Temperatures fluctuate seasonally between 12 °C and 24 °C (Coetzee 1983). Since the lagoon is shallow, wind-generated waves readily disturb bottom sediments, resulting in high turbidity levels, especially in the shallower northern section of the lagoon (pers. obs.).

The sediments of the lagoon vary from glutinous mud (<9% sand) to fine and medium sands with negligible mud fractions (Willis 1985). Sandy sediments are found along the shores, where the mud fractions are removed by wave action, as well as in larger areas closer to the sea, where sand is deposited tidally when the mouth is open

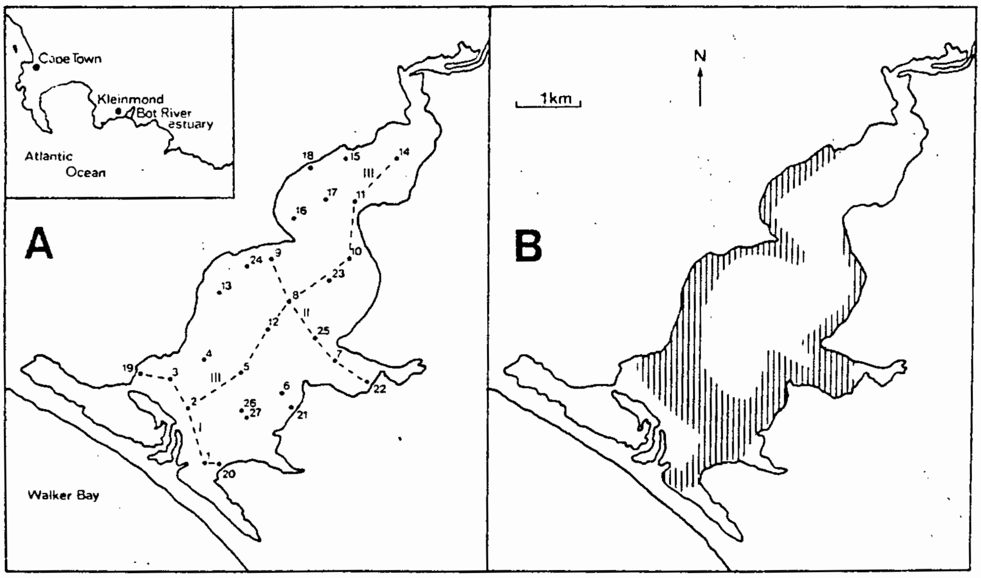

Fig. 1. Map of the Bot River estuary, showing sample sites (A), and *Ruppia maritima* distribution in April 1983 (B). The transects in Figures 2, 3 and 4 are indicated by I, II and III.

(Theron *et al.* 1981), and by aeolian transport from the coastal dunes. The muddy sediments are found mainly in the deeper, central areas of the lagoon, with an increase in the sand fraction as depth decreases (Fig. 9). Mud and clay may also enter via the Bot River and are deposited by flocculation at the head of the estuary (Willis 1985).

The major aquatic macrophyte in the system is *Ruppia maritima*, which colonizes the estuary in a wide band along the edges, leaving a large central area clear of any macrophytes (Fig. 1B). At the time of the surveys, no *Potamogeton pectinatus* was present, although this plant had been present earlier (Bally *et al.* 1985). It seems that the *Potamogeton* populations had been smothered by flood-borne sediments in February 1981, while high salinities, persisting after an artificial opening in October 1981, prevented this species from re-establishing itself (pers. obs.). Colonization by *Ruppia* seems to be controlled mainly by light attenuation due to turbidity and, since this fluctuates considerably, both spatially and temporally, colonization depth varies, but seldom exceeds 2,9 m (Koop *et al.* 1983).

MATERIALS AND METHODS

Collection of macrofauna samples

Sediment samples were taken from the Bot River estuary at 22 sample sites in December 1982 (Fig. 1A, 1–22) and from 27 sites in May 1983 (Fig. 1A, 1–27). The estuary had then been closed since December 1981, after an artificial breaching in October 1981. The samples were taken by SCUBA divers with a rigid plastic box (15 cm × 11 cm × 9 cm; 165 cm^3 cutting area) with a removable lid. The inverted box was pushed into the sediment to a depth of 9 cm, whereafter the lid was inserted to prevent the loss of sediment while the box was brought to the surface. Duplicate samples were taken at each sample site, placed together in plastic bags, and subsequently sieved through 1 mm mesh sieves. The remaining material was preserved in 5% formalin and sorted by hand under a binocular microscope. All animals were removed, identified and counted. A drawback of this sampling method was the unavoidable underestimation of *Callianassa kraussi* abundances, due to their deep burrows. At most sites all other species were confined to the top few centimetres of sediments due to the presence of an anoxic layer close to the surface.

Representative numbers of animals of the more abundant taxa were dried to constant mass in an oven at 60 °C and weighed to determine average dry mass (Table 1). These data were used to calculate biomass estimates for all sites in both December and May. Shells of molluscs were removed with dilute HCl prior to drying. Insufficient numbers of 12 taxa were found to allow their dry mass determinations. Abundances are given as numbers per m^2, while biomass estimates, calculated from standing stocks and dry mass, are given as mg dry mass per m^2.

Statistical analyses

The numerical data of abundance at each sample site were used to investigate similarities between the sites. The Bray–Curtis similarity measure (Bray & Curtis

Table 1

Masses of benthic macrofauna used in the calculation of biomass estimates of standing stocks in the Bot River estuary

Species	Number weighed	Dry mass per animal (mg)
Amphipoda		
Corophium triaenonyx	165	0,048
Melita zeylanica	508	0,250
Isopoda		
Exosphaeroma hylecoetes	245	1,534
Cyathura estuaria	262	0,905
Tanaidacea		
Apseudes digitalis	2 296	0,092
Anomura		
Callianassa kraussi	20	4,593
Polychaeta		
Ceratonereis erythraeensis	354	0,140
Prionospio pernana	418	0,086
Capitella capitata	348	0,084
Oligochaeta	109	0,038
Mollusca (shell free)		
Arcuatula capensis	342	16,490
Hydrobia sp.	342	0,287
Insecta		
Dipteran larva sp. 1	5	1,257
Chironomid larva	4	0,471
Actiniaria	432	0,098
Turbellaria	10	0,077

1957), which is abundance-weighted was used to construct similarity matrices. These, in turn, were analysed using classification and ordination techniques (Velimirov *et al.* 1977, Field *et al.* 1982). For this procedure, the data were log-transformed and similarities then summarized in dendrograms and two-dimensional ordinations (not given here). Dendrograms graphically depict the percentage similarities between sample sites. Classification was performed by the group-average sorting method (Clifford & Stephenson 1975), which joins groups of samples together at the average level of similarity between all members of both groups (Field *et al.* 1982).

After sample sites had been grouped by similarity, the species responsible for these groupings had to be determined. This was done by using information statistic tests on the numerical data to determine which species differ most between any two groups of samples (Field 1969, Field *et al.* 1982). In this way, taxa typical of similar sample site groups could be found. All computing was done on the Sperry-Univac 1100 Series computer at the University of Cape Town.

Collection of meiofaunal samples

Meiofauna was collected from 10 sites around the Bot River estuary, using a standard corer. At each site, five samples were collected, each sample consisting of four cores thoroughly mixed together, subsampled and then preserved in formalin. Extraction was carried out using a modified Oostenbrinck meiofauna extractor (Fricke 1979), whereafter the animals were stained with rose bengal and counted under a binocular microscope. Abundances are given as animals cm^{-3}.

RESULTS

Species composition, abundance, and biomass

The results of the two surveys are summarized in Tables 2–4 and Figures 2–8. Table 2 shows a total of 25 species recorded in the two surveys, 18 in December and 23 in May. Two of the species found in December were absent in May while 7 of the 23 species recorded in May were absent in December. The Polychaeta were the biggest group with 5 species, while other important groups were the Mollusca (4 species), the Isopoda (4 species) and the Amphipoda (3 species). Despite the increase in the number of species from December to May, the total mean abundance of 5 915 animals m^{-2} in December decreased by 16% to 4 967 animals m^{-2} in May. Maximum numbers of animals (18 604 m^{-2}) were found at site 9 (Fig. 1A) in December and at site 20 (17 031 m^{-2}) in May. Total mean biomass, however, decreased by 48%, from 8,29 g dry mass m^{-2} in December, to 4,28 g dry mass m^{-2} in May. The maximum biomass (91,63 g dry mass m^{-2}) was again found at site 9 in December, while the maximum in May (29,24 g dry mass m^{-2}) was found at site 26.

Table 3 gives the percentage abundance and biomass of the 11 most common species in the estuary (all present at more than 50% of the sample sites in either December or May, or both). Only 6 species were present at more than half the sample sites in both months. The dominance of these species is shown by the fact that they formed between 93 and 98% of the total abundance and biomass in both December and May.

The discrepancy between the reduction in total mean abundance (16%) and biomass (48%) from December to May, can be attributed mainly to changes in the abundance of two species: the tanaid, *Apseudes digitalis*, and the bivalve, *Arcuatula capensis*. From Table 3 it is clear that *Apseudes digitalis* was numerically dominant in May (40,9% of total abundance), while *Arcuatula capensis* formed 76% of the biomass of both months. *Apseudes digitalis* increased by 57,5% from December to May, which reduced the effect of the large decreases in abundance of most other common species on the total mean abundance. On the other hand, the reduction in abundance of *Arcuatula capensis* of 47,8% was the main reason for the large drop in biomass from December to May.

In December the most abundant species was the polychaete, *Capitella capitata*, forming 16% of the total mean abundance, while *Apseudes digitalis* formed 15%. *Apseudes digitalis* reached the highest number at one site (site 9) of 6 363 animals m^{-2} in December, while *Capitella capitata* reached maxima of 4 619 animals m^{-2} at sites 18 and

Table 2

Species composition of the benthic macrofauna of the Bot River estuary. The abundance, A (animals m^{-2}), biomass, B (mg dry mass m^{-2}) and the number of sites at which each species was present, are given

Species	December Sites	December A	December B	May Sites	May A	May B
Amphipoda						
Corophium triaenonyx	13	439,23	21,09	11	44,85	2.15
Melita zeylanica	17	679,55	169,55	15	203,19	50,69
Orchestia ancheidos	0	0	0	1	2,26	—
Isopoda						
Exosphaeroma hylecoetes	17	460.68	738,64	19	211,04	323,73
Cyathura estuaria	16	485.91	438,36	15	116,70	105,58
Panathura sp.	0	0	0	5	19,07	—
Synidotea sp.	0	0	0	2	2,22	—
Tanaidacea						
Apseudes digitalis	11	863,64	79,55	21	2 031,26	187,06
Anomura						
Callianassa kraussi	5	67,14	308,36	3	6,74	30,96
Polychaeta						
Ceratonereis erythraeensis	13	514,64	72,18	14	190,78	26,77
Prionospio pernana	17	660,95	56,82	15	271,59	23,34
Capitella capitata	13	964,23	81,27	13	395,04	33,10
Desdemona ornata	6	45,09	—	2	3,37	—
Lumbrinereis tetraura	1	2,77	—	1	1,11	—
Oligochaeta	2	11,05	0,42	1	279,45	10,62
Mollusca						
Arcuatula capensis	10	380,95	6 281,94	15	198,70	3 276,62
Hydrobia sp.	0	0	0	20	520,74	149,45
Pulmonate sp.	0	0	0	1	1,11	—
Tomichia sp.	0	0	0	1	2,26	—
Insecta						
Dipteran larva sp. 1	1	5,23	6,59	1	11,22	14,11
Dipteran larva sp. 2	0	0	0	1	1,11	—
Chironomid larva	5	53,82	25,32	2	3,37	1,59
Actiniaria	10	145,68	14,27	18	450,00	44,08
Nematoda	7	110,45	—	0	0	0
Turbellaria	5	24,32	1,88	0	0	0
Total no. species		18			23	
Mean totals		5 915.33	8 296,24		4 967,18	4 279,85
Standard errors		996,63	4 085,54		842,80	1 312,86

22. Overall, the abundance in December was not dominated by any particular species, compared to May, when *Apseudes digitalis* formed 41 % of the total mean abundance. The next most abundant species in May, the gastropod *Hydrobia* sp., formed only 11 % of the total mean abundance, although it was completely absent in December. *Apseudes digitalis* reached a maximum abundance of 7 787 animals m^{-2} at site 9 in May.

Table 3
The percentage abundance (A) and biomass (B) composition of the 11 most common species of benthic macrofauna of the Bot River estuary

Species	December 1982		May 1983	
	% A	% B	% A	% B
Corophium triaenonyx	7,43	0,25	0,90	0,05
Melita zeylanica	11,49	2,04	4,09	1,18
Exosphaeroma hylecoetes	7,79	8,90	4,25	7,56
Cyathura estuaria	8,21	5,28	2,35	2,47
Apseudes digitalis	14,60	0,96	40,89	4,37
Ceratonereis erythraeensis	8,70	0,87	3,84	0,63
Prionospio pernana	11,17	0,68	5,47	0,55
Capitella capitata	16,30	0,98	7,95	0,77
Arcuatula capensis	6,44	75,72	4,00	76,56
Hydrobia sp.	0	0	10,48	3,49
Actiniaria	2,46	0,17	9,06	1,03
Totals	94,95	95,85	93,28	98,66

Total mean biomass was made up mostly by *Arcuatula capensis* in both December (75,7 %) and May (76,6 %). The maxima reached by this species were 87,94 g dry mass m^{-2} at site 9 in December, and 25,99 g dry mass m^{-2} at site 26 in May.

Transects

Figures 2, 3 and 4 illustrate the changes in biomass along three transects in the Bot River estuary. In all three figures it is clear that there are large fluctuations in biomass in the estuary. Nevertheless, there is a distinct pattern which occurs in both December and May. Sites along the shore and in the central area (clear of *Ruppia*) have the lowest biomass levels, while biomass increases manyfold in the areas colonized by *Ruppia maritima*. The very low biomass at site 19 in May (Fig. 2) could be due to fresh water input from the Lamloch River, which flows into the estuary at this site.

On the basis of the transects, it seems that the estuary can be divided into three zones, viz., a Littoral zone, including sites along the shores, a *Ruppia* zone, including sites in the macrophyte beds, and a Deep zone, including sites in the central, deeper areas without macrophytes. Figure 5 illustrates the changes in average biomass in these three zones in the two months. The *Ruppia* zone clearly had the largest invertebrate communities in both December and May. Although the average biomass in both the *Ruppia* and the Littoral zones decreased from December to May, it increased by 43 % in the Deep zone. This was due mainly to an increase in *A. capensis* abundance at these sites in May.

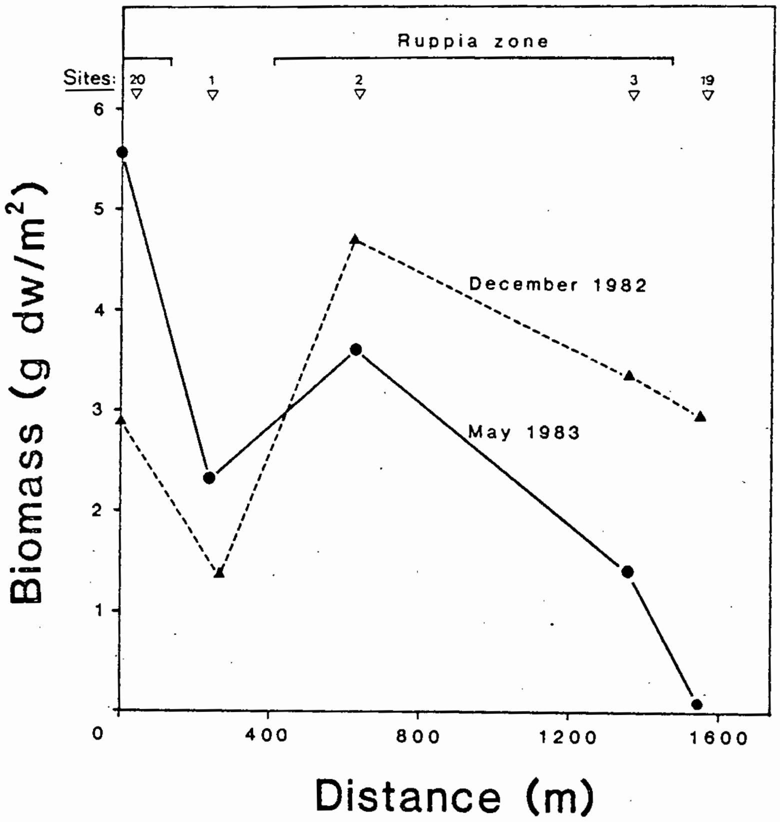

Fig. 2. Fluctuations of total biomass (g dry mass m^{-2}) in a transect across the Bot River estuary in December 1982 and May 1983.

Similarities between sites

Similarities between sample sites, analysed on the basis of the numerical abundance of every species at each site, are illustrated in Figures 6, 7 and 8. Examination of the clustering of the December sites in Figure 6 shows that they separate into two main clusters at the 40% similarity level: a cluster of deep sites, and a cluster of shallow sites. These two clusters, in turn, can be divided into two groups each at the 50% similarity level, resulting in four clusters of varying intra-group similarities. The cluster of deep sites can be clearly divided into sites colonised by *Ruppia maritima* (*Ruppia* zone) and sites in the central, clear area of the estuary (Deep zone). The sites within these two groups all show a high level of similarity, with the majority clustering above 70%. The second group of shallower sites, on the other hand, can be divided into those sites which moved with the changes in the water-level to remain within 30 m of the shoreline (Littoral zone) and fixed shallow sites on sandy bottoms (Sand zone). The intra-group similarities of the Littoral and Sand zones (50–60%) are not as high as those in the first two zones.

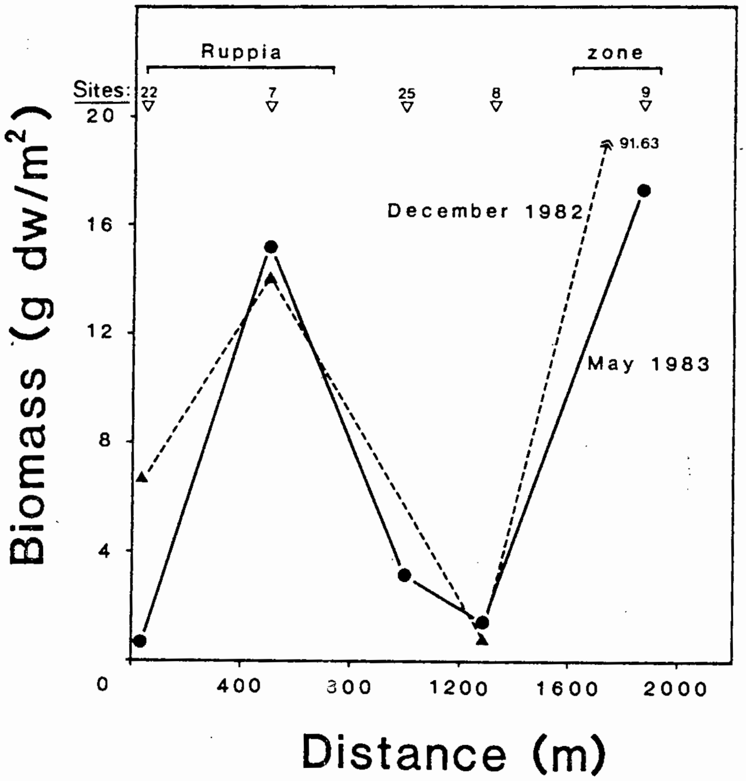

Fig. 3. Fluctuations of total biomass (g dry mass m^{-2}) in a transect across the Bot river estuary in December 1982 and May 1983.

In figure 7, the clustering of the May sites, the same four groups are discernible. The *Ruppia* and Deep zones are again clustered clearly at high similarities (above 60 %), while the clusters of the Littoral and Sand zones are less distinct and have very variable intra-group similarities. This variability is especially evident in the Littoral zone cluster.

Figure 8 illustrates the similarities obtained between all the sites when the data from December and May were combined and analysed together. This was done to investigate whether any seasonal variation exists. Seasonality would be shown by clusters of sites in December separated from the same sites in May. Careful examination of Figure 8 shows that although a few sites cluster together in separate months, the four zones are still distinctly discernible, but they are now grouped into six clusters due to the division of the Deep and Littoral zones into two clusters each. Neither the Littoral nor the Deep zone clusters are clearly divided into separate months, however, and the division of the zones seems to be an artefact of the clustering technique. It seems therefore, that there is little seasonal variation and that spatial variation is the main trend, since at lower similarities the four zones are clearly discernible, irrespective of sample month.

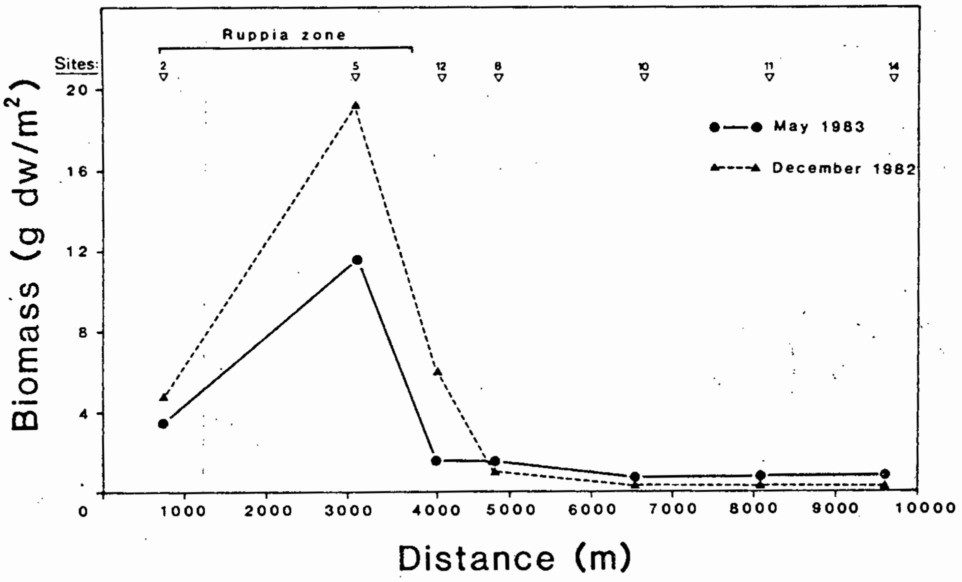

Fig. 4. Fluctuations of total biomass (g dry mass m^{-2}) in a transect along the length of the Bot River estuary in December 1982 and May 1983.

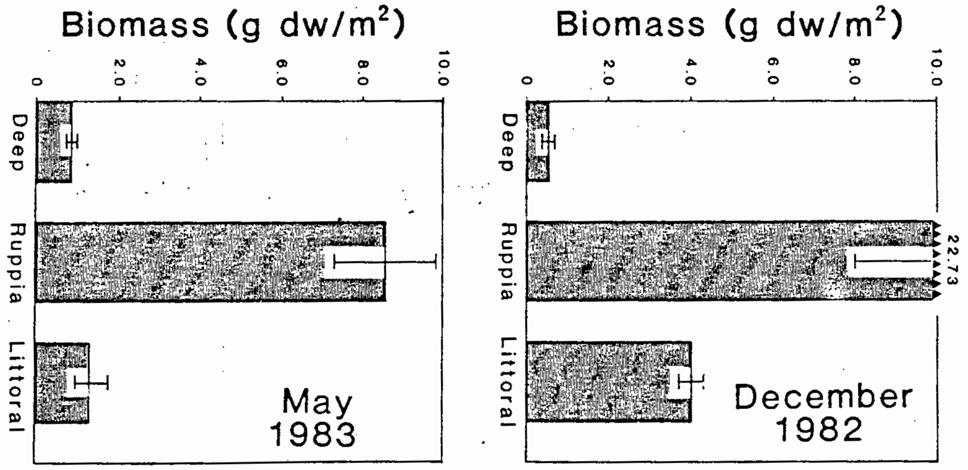

Fig. 5. Variations in average biomass (g dry mass m^{-2}) at sites in three zones in the Bot River estuary. Vertical bars represent one standard error of the mean.

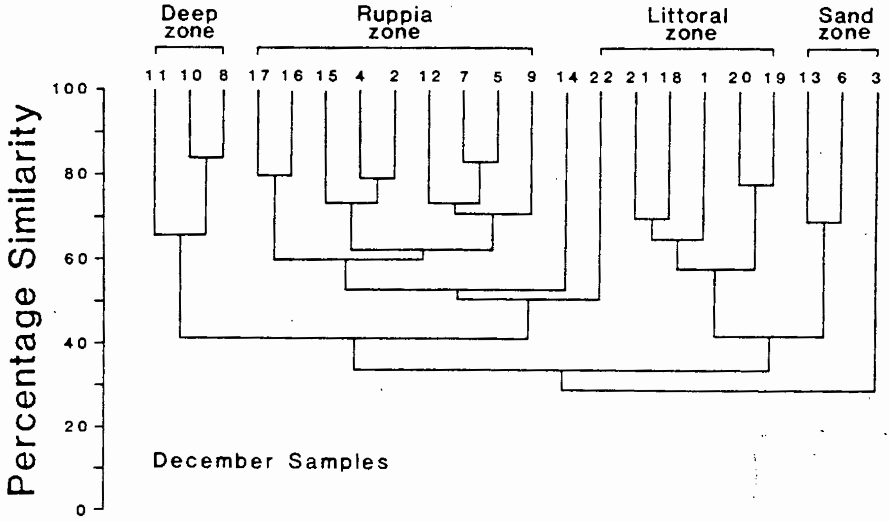

Fig. 6. Dendrogram showing the similarities between all sites sampled in the Bot River estuary in December 1982.

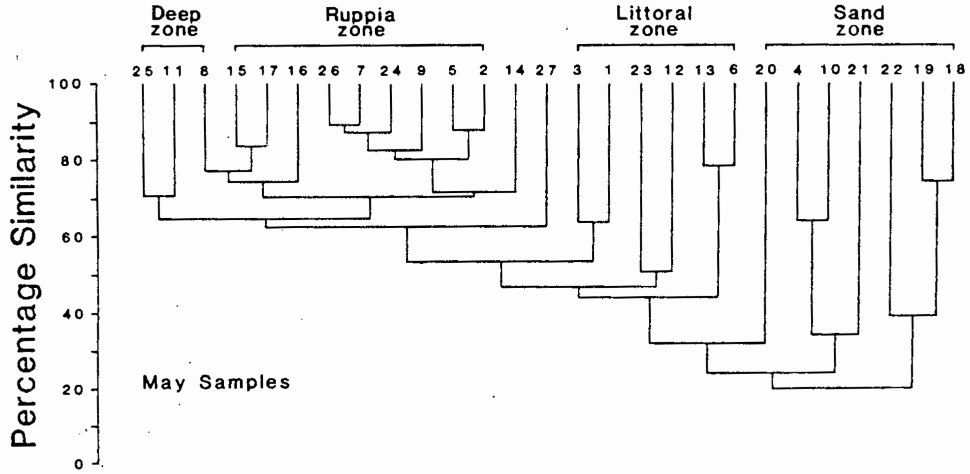

Fig. 7. Dendrogram showing the similarities between all sites sampled in the Bot River estuary May 1983.

Characteristic species

Once they had been identified, it was important to determine whether the zones only differed in terms of the abundance of species present, or whether certain species were characteristic of a zone. This could also lead to a separation between sample sites. The data for December and May were pooled and analysed using information statistic

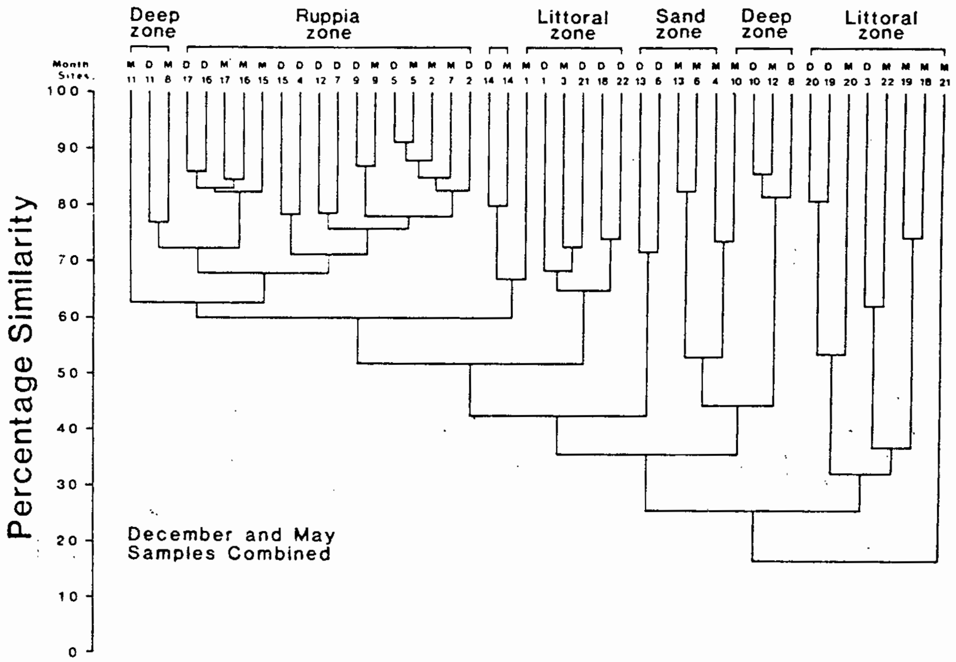

Fig. 8. Dendrogram showing the similarities between all sites combined, for December 1982 and May 1983.

tests on the basis of the frequency of occurrence of species in each zone. Since the number of sample sites in each zone differed markedly, direct comparisons were not possible. The sites were therefore divided into those in deep areas (*Ruppia* and Deep zones) and those in shallow areas (Littoral and Sand zones) on the basis of the grouping in Figure 6. Table 4 gives the taxa that differed significantly at the 1% and 5% levels between the two areas. Four species were characteristic of the deeper areas, while in the shallower areas five species had significantly higher abundances.

Meiofauna

Figure 9 shows the meiofaunal densities, as well as their variation with percentage sand fraction in the estuary. The meiofauna is surprisingly poor, both in terms of diversity and density. Only three groups, the platyhelminths, nematodes and oligochaetes were present in most samples. Many samples also contained a few juvenile macrofaunal amphipods. Harpacticoid copepods, chironomids and polychaetes were also found, all in very few samples. The harpacticoids were clearly marine imports to the system since they were found only in a single sample within the open mouth. There seems to be a trend of greater density and diversity with increased sand fractions in the sediments.

Table 4

Total abundances of species differing significantly between sites in deep areas (*Ruppia* and Deep zones) and in shallow areas (Littoral and Sand zones). Significance levels are shown by ★ = 5 %, ★★ = 1 %

Species	Deep areas	Shallow areas
	(total numbers at all sites)	
Characteristic of deep areas		
Melita zeylanica	15727	2376 ★
Apseudes digitalis	54663	545 ★★
Arcuatula capensis	10515	534 ★★
Actiniaria	11543	1114 ★★
Characteristic of shallow areas		
Exosphaeroma hylecoetes	2608	11410 ★
Callianassa kraussi	0	1062 ★★
Capitella capitata	2030	29363 ★
Oligochaeta	0	6606 ★★
Chironomid larvae	0	101x ★★

DISCUSSION

Species composition, abundance, and biomass

Koop *et al.* (1983) in their survey of the Bot River estuary in April 1979, found a total of 17 species of benthic macrofauna along 5 transects of the vlei. Although this is close to the 18 species recorded in our study in December 1982, there are important differences in the species composition and abundances between the two studies. The maximum biomass of 8,13 g dry mass m^{-2} recorded in their study falls far short of the maximum of 91,63 g dry mass m^{-2} found by us, but is close to the mean biomass for December 1982 of 8,29 g dry mass m^{-2}. In the study of Koop *et al.* (1983), the numerically dominant species was *Hydrobia*, while the isopod *Exosphaeroma hylecoetes* had the highest biomass. In our surveys, however, *Apseudes digitalis*, which was relatively rare in the 1979 survey, was numerically dominant, while *Arcuatula capensis* formed 76 % of the biomass in both months. *Hydrobia*, in fact, was absent in our December survey. One reason for these large differences could be the fact that Koop *et al.* (1983) only sampled the shallower regions of the vlei (up to 250 m from the shore), compared to our general survey (Fig. 1A). Further differences between the two studies are the absence of *Assiminea, Nephtys zeylanica* and *Palaemon pacificus* from our samples, while 4 polychaetes and 7 other species were not found in the 1979 survey.

Koop *et al.* (1983) noted that a feature of the benthic macrofauna of the Bot River estuary, when it is compared to other estuaries, is its low diversity and biomass. This is patently clear from the present study, considering that only 11 species formed up to 98 % of the total biomass of the estuary. Low diversities are further evident when the

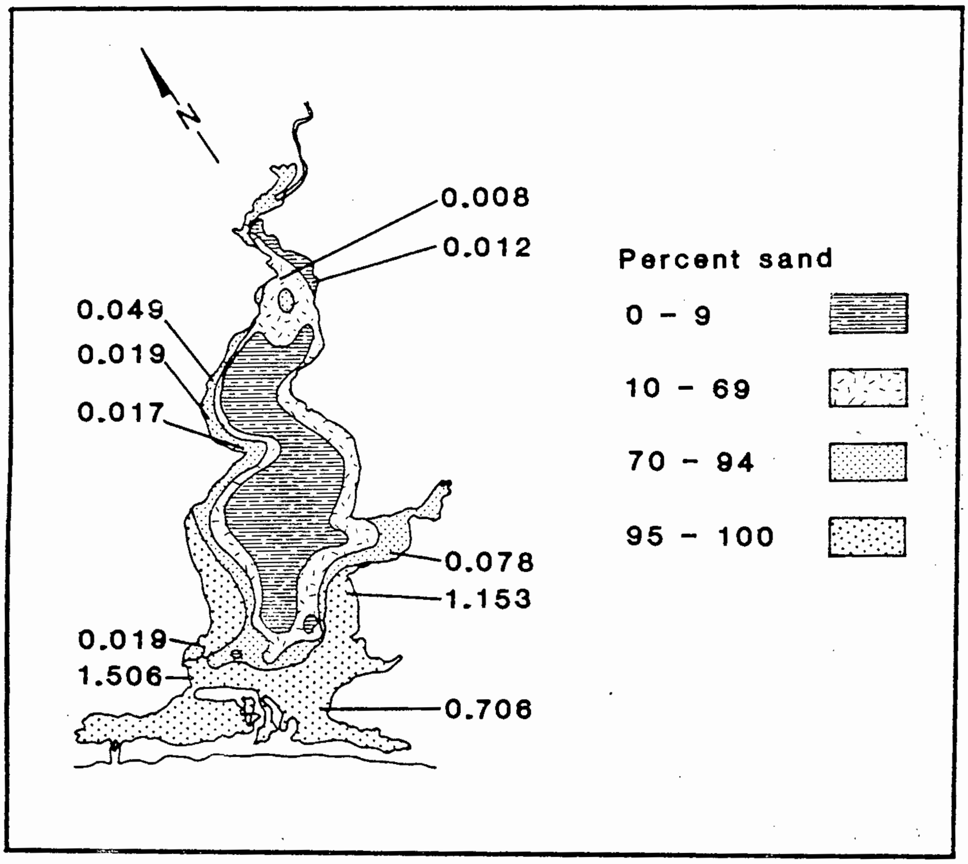

Fig. 9. Map of the Bot River estuary showing the densities of meiofauna (numbers cm^{-3}) in relation to the percentage sand fraction in the sediments (Sedimentology after Willis 1985).

species list is compared to that provided by Day (1981) in a summary of work published on many South African estuaries. A distinction has to be made, however, between tidal and blind estuaries (Day 1951) in the consideration of the abundances and diversities of benthic macrofauna. Permanently open tidal estuaries, for example the Knysna estuary with 319 species (Day *et al.* 1952, Day 1967), have very high benthic diversities. Blind estuaries, for example the Klein River estuary with 134 species (Scott *et al.* 1952), on the other hand, have lower diversities due to the reduction of recruitment from the sea (Day 1964). The mouth of the Bot River is almost permanently closed (Koop 1982) and is only artificially breached every few years (Branch *et al.* 1985). Recruitment is consequently at a minimum and breaching disturbs the system frequently. This is reflected by the low benthic diversity. Only 27 species have been found in the Bot River estuary.

In this respect the estuary resembles a coastal lake, which is permanently cut off from the sea. Allanson (1981) has synthesized published data of four southern African coastal lakes: Swartvlei and lakes Poelela, Nhlange and Sibaya. Despite the large environmental differences, the species composition given here for the Bot River estuary (Table 2), compares well with a checklist of 37 species given for the four systems, while biomass figures for Lake Poelela (Boltt 1975) and Lake Sibaya (Hart 1979) correspond with that found in the Bot River estuary. Furthermore, the fauna of Lake Sibaya has been described as a relict estuarine fauna (Allanson *et al.* 1966). It is obvious therefore, that the benthic macrofauna of the Bot River estuary is impoverished in comparison with the densities and diversities found in tidal estuaries, and that as an almost permanently closed estuary, its faunal composition strongly resembles that of a coastal lake.

Distribution

Cluster analyses indicate that the estuary can be divided roughly into two main zones, viz., the zone included by the *Ruppia* band, and the zone shallower than the macrophytes. These two zones can be further divided into the *Ruppia* and Deep zones, and the Littoral and Sand zones, respectively. The *Ruppia* zone is the most clearly defined and is characterized by high levels of similarity between sample sites, as well as high invertebrate biomass. One of the characteristic species of this zone is *Arcuatula capensis*, which forms the largest part of the standing stocks of the lagoon. The importance of the macrophytes to the system is clearly illustrated by the marked difference in the average biomass values from sites in the Deep and *Ruppia* zones. Generally, however, these two zones are characterized by the same species.

The distinction between the two zones of the shallow areas, the Littoral and Sand zones, is not as clear. Similarities between the sample sites are not as high as in the first two zones and, in some cases, the sample sites cluster in the 'wrong' zones. This could be due to the fact that the physical distinctions between these two zones are not as clearcut as is the case in the former two zones. All the sample sites in the shallow areas are on relatively sandy bottoms (Fig. 9), and the only real difference between the two zones is that the sites in the Littoral zone are affected by the 'drawdown' in the estuary; when the water-level decreases, large areas of the bottom are exposed, which are then inundated again when water-levels rise. Since the sample sites in the Littoral zone move with the water-level in order to remain within 30 m of the shoreline, they reflect the changes in the fauna due to the variation in water-levels. This could result in the observed differences in similarities between sample sites of the Littoral and Sand zones.

The distinction between the deep and shallow areas is further indicated by the different characteristic species of the two areas. Five species were characteristic of the shallow areas, and four of the deep areas. The 'preferences' of the species can probably be accounted for by the presence or absence of *Ruppia* and/or differences in sedimentology between the two areas. *Arcuatula capensis* and the anemones, for example, are associated with the macrophyte beds, while *Callianassa kraussi* constructs deep burrows in the shallow, sandy areas only.

The cluster analyses also show that, in general, faunal composition changes more between sample sites than seasonally. This was to be expected, since temperature was the only real physical change from December to May, while other factors, like salinity, remained fairly constant. The lack of contact with the sea probably also affects seasonality. In open estuaries, the majority of the larval invertebrates are presumably recruited seasonally from the sea (Day 1964), but if the mouth is permanently closed, this seasonal effect is eliminated.

Koop *et al.* (1983) reported that they had found no marked differences in species composition between their transects, and that major species were found at all sites. Our study, however, has shown that the species composition does vary between sample sites and that although most species may be widely distributed, the majority of the dominant species could be seen as characteristic of a particular zone. The uniformity found by Koop *et al.* (1983) was attributed to the well-mixed nature of the water of the estuary, but it is obvious from the present study that sharp boundaries between physical and biotic factors exist, for example between *Ruppia*-dominated and clear areas, or muddy and sandy sediments. These differences result in changes in species composition and distribution of the benthos. It should be noted, however, that Koop *et al.* (1983) completed their survey two and a half years after mouth closure and after 18 months of low salinities in the lagoon (Bally & McQuaid 1985). This contrasts with our study, which was carried out during a period of relatively high salinities, due to poor winter rainfall after the mouth had closed in December 1981, 12 months prior to the December 1982 survey. Large changes in the abundance of invertebrates can also be expected when the mouth is artificially opened, associated with the decline of weeds (as Davies (1982) has recorded at Swartvlei).

Meiofauna

Dye & Furstenburg (1981) report that estuaries are usually rich in meiofauna and that the average density for South African estuaries is approximately 100 animals cm^{-3}. The Bot River estuary, in comparison, has meiofaunal densities ranging from 0,008 cm^{-3} to a maximum of 1,506 cm^{-3}. Furthermore, 52 genera have been identified from the Swartkops estuary (Dye & Furstenburg 1977), compared to the 7 groups found in the Bot River estuary. The poor meiofaunal density and diversity can probably be related to the sediment characteristics of the estuary, shown by the trend of increased density and diversity with increased sand fraction in the sediments. The reason for this lies in the size of the interstitial spaces available in the various grades of sediment. The higher the mud fraction, the less space there is, and organisms become physically excluded from the interstices. The reduction in interstitial space also results in a decrease in the porosity of the sediment, thereby reducing interstitial circulation and increasing the tendency of the sediments to become anoxic. These two factors, or a combination thereof, are the most probable causes for the poor meiofauna of the Bot River estuary and the large sizes of the individuals in comparison with sandy beach meiofauna. The large sizes are a consequence of the reduced interstitial spaces, since organisms have to be large enough to be able to displace particles when moving through the sediment.

In conclusion, it seems clear that, in terms of the benthic fauna, the Bot River estuary is a blind estuary of extremely low species abundances, diversities and biomass. Contact with the sea has been reduced to the extent that the estuary faunistically resembles a coastal lake, and periodic artificial opening of the mouth prevents the system from stabilizing.

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CHAPTER TWO

Breaching the mouth of the Bot River estuary, South Africa: impact on its benthic macrofaunal communities.

ABSTRACT

The changes occurring in the benthic macrofaunal communities of the Bot River estuary, after an artificial opening of its mouth on 29 June 1983, are presented and the possible short- and long-term effects of this disturbance on their ecology are described. Surveys of the benthos conducted in December 1982 and May 1983, before the mouth-breaching, are compared with similar surveys afterwards: in October 1983, a week prior to reclosure of the estuary, and three months later, in January 1984. The total number of species decreased from 23 in May to 15 in October; however only those species which contributed less than 5% to the total biomass in May were lost. The largest reductions in species abundance and biomass were related to the collapse of the macrophyte, *Ruppia maritima*, which is intolerant to high salinities. The species which were reduced most were *Arcuatula capensis*, *Melita zeylanica* and *Cyathura estuaria*. These species were adversely affected by the loss of their attachment substrate (*Arcuatula capensis*), and possibly by increased predation pressure due to the reduction in macrophytic cover. In the areas not colonised by *Ruppia*, the dominant species exhibited an increase in densities and biomass. This phenomenon may be attributed to tidally-induced currents which existed while the mouth was open and which transported nutrients from the decaying macrophytes in the shallows to communities in the deeper waters. This assumption is strengthened by the fact that densities in the deeper areas returned to their usual low levels once closure of the mouth had eliminated these currents.

In the longer term, breaching of the mouth seems to influence

species composition and diversity. After an extended period of separation from the sea, the benthic macrofauna appears to develop towards that of a coastal lake. The unpredictable, catastrophic disturbance caused by opening the mouth, however, interrupts this development by eliminating the "lagoonal" species and thereby also maintaining very low species diversities.

INTRODUCTION

The Bot River Estuary, between Kleinmond and Hawston on the southwest coast of South Africa (Fig. 1), is unlike many South African blind estuaries (*sensu* Day 1981), since the sandbar which separates it from the sea is very rarely breached naturally. Willis (1985) has given a detailed description of the estuary, while management options based on completed research may be found in Branch *et al*. (1985). Flood levels are reduced by overflow to the neighbouring Kleinmond estuary, which prevents the mouth from breaching, but also reduces salinities, due to dilution by freshwater input from the river. Very low salinities may in turn lead to fish mortalities (Bennett 1985) and rapid growth of the macrophytes *Ruppia maritima* and *Potamogeton pectinatus* (Branch et al. 1985). These effects, as well as the flooding of neighbouring properties, are undesirable to recreational users and local landowners, who clamour for the artificial breaching of the mouth. This event introduces a tidal influence to the estuary, raising salinities and preventing further flooding. Unfortunately, it also has an enormous ecological impact on the system by reducing water levels sufficiently to expose most of the colonising *Ruppia maritima*. The effects of such a catastrophic perturbation have not been monitored in any South African estuary and the opportunity to do so presented itself when, on 29 June 1983, three weeks after a survey of the benthic macrofauna was fortuitously completed, the Bot River estuary was artificially opened at Sonesta (Fromme 1985).

Longshore currents in the sea subsequently closed the mouth three months later, eliminating the tidal regime, but a survey was

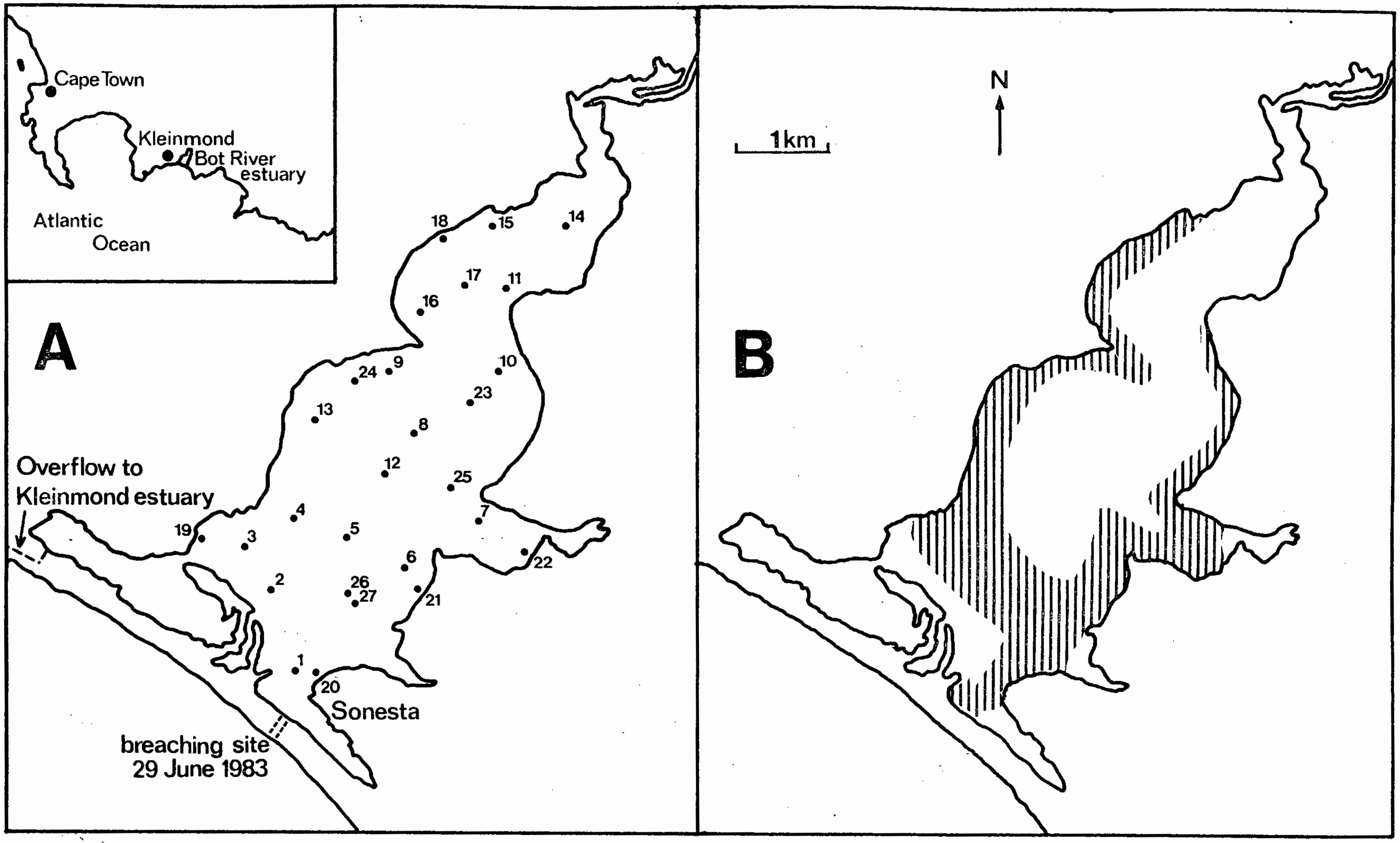

Figure 1: Map of the Bot River estuary, showing sample sites (A) and *Ruppia* distribution in April 1983 (B), two months before the mouth was opened at Sonesta (29 June 1983).

undertaken in October, a week before the mouth closed completely, to determine the effect of the breaching and the consequent tidal influences on the benthic invertebrates. A third survey was conducted three months later, in January 1984, to assess recovery of the fauna. This paper reports the changes which occurred in the benthic macrofaunal communities due to the mouth-breaching, and interprets possible short- and long-term effects of such unpredictable catastrophic disturbances on the ecology of estuarine benthos.

MATERIALS AND METHODS

Measurement of depths and salinities

Depth recordings were obtained from a continuous-recording depth gauge maintained by the Department of Water Affairs and have been converted to heights above MSL (Fig. 2). Salinity measurements were made at each sample site with a hand-held refractometer (American Optical Corporation), which gives sufficient resolution (0.5‰) for the large salinity fluctuations in the estuary.

Collection of benthic faunal samples

Surveys of the benthic macrofauna were undertaken in December 1982 and May 1983, before the breaching on 29 June 1983, while the effects of the breaching and subsequent recovery were assessed by surveys in October 1983 (a week before mouth closure) and three months later, in January 1984. A minimum of 22 and maximum of 27 sites were sampled during these months (Fig. 1A), depending on water levels in the estuary. Faunal samples were taken by SCUBA divers using a box corer (15cm x 11cm x 9cm; 165cm^2 cutting area), and were processed using the methods given by De Decker and Bally (1985) to yield species abundances and biomasses.

Statistical analyses

Numerical classification of the abundances at each site was used to investigate possible changes in the zonation patterns of faunal species assemblages. Between-site similarities were calculated

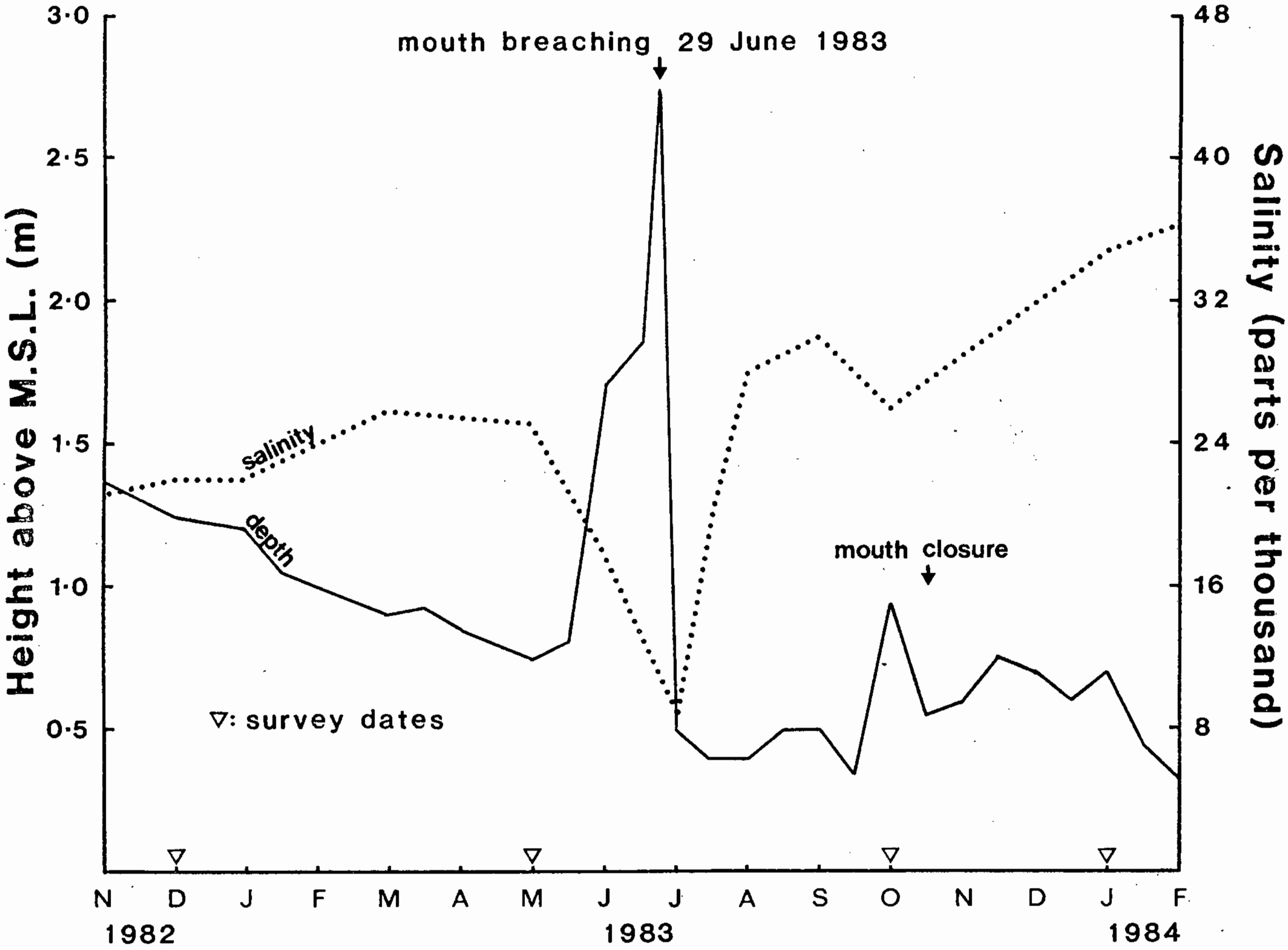

Figure 2: Variations in depth (height above M.S.L. in metres) and salinity (parts per thousand) in the Bot River estuary from November 1982 to February 1984.

using the Bray-Curtis abundance-weighted similarity measure (Bray & Curtis 1957) on log-transformed data (Yij=log(Xij+1)). The similarity matrix was summarised in dendrograms, using the method of group-average sorting (Clifford & Stephenson 1975, Field et al. 1982), and in two-dimensional ordinations, by non-metric multi-dimensional scaling (Kruskal & Wish 1978). Ordination is a technique complementary to classification, in which the relationships between the sample sites are represented as distances on a plane, in a manner which preserves as much as possible of the total amount of variation in the multi-dimensional system. Sites with similar species composition are grouped in this plane into clusters which correspond to those revealed by the dendrogram (Velimirov et al. 1977). In these ways, possible changes in the spatial patterns of community structure following the mouth-breaching were investigated, by analysing species abundance data from the surveys directly before and after the event (May 1983 and October 1983 respectively). In the first instance, these months were analysed separately. The data from May and October were then pooled, to test if the spatial groupings identified when the mouth was closed, were still discernible. The results were compared with those from a similar analysis undertaken by De Decker and Bally (1985), when the mouth had been closed for some time.

RESULTS

Water levels behind the sandbar fluctuate from mean sea level (MSL) to a maximum of +2.7m above MSL, depending on freshwater input and evaporation (Fig. 2). When the mouth is breached, the water level drops rapidly to just above MSL and the salinity increases to that of seawater (approximately 35‰). The estuary is colonised along its fringe by Ruppia maritima, which is the major macrophyte in the system (Fig. 1B). Verhoeven (1979) reports that European Ruppia maritima communities have a maximum salinity tolerance of 24.4‰ and consequently, those in the Bot River estuary that are not stranded during the drop in water levels, do not survive an increase in salinity of such magnitude. Recovery of the macrophyte commences only once the mouth is closed again and salinities are decreased by freshwater input (pers. obs.).

De Decker and Bally (1985) have identified four benthic macrofaunal zones in the Bot River estuary, viz, a zone dominated by Ruppia maritima (Ruppia zone) the central area, free of macrophytes (Deep zone), a shallow zone fringing the shore (Littoral zone) and a more diffuse region characterised by sandy substrata (Sand zone). Since the fourth zone constitutes a less distinct zonation of minor importance, it will be ignored in the following discussion. This spatial pattern provided a useful framework for investigating the changes that occurred in the benthic macrofauna after the mouth was breached. For this purpose, the Ruppia zone is represented here by 8 sites (2,3,5,7,9,13,15 and 16), the Deep zone by 5 sites (8,10,11,12 and 17) and the Littoral zone by 5 sites (18 to 22), following the

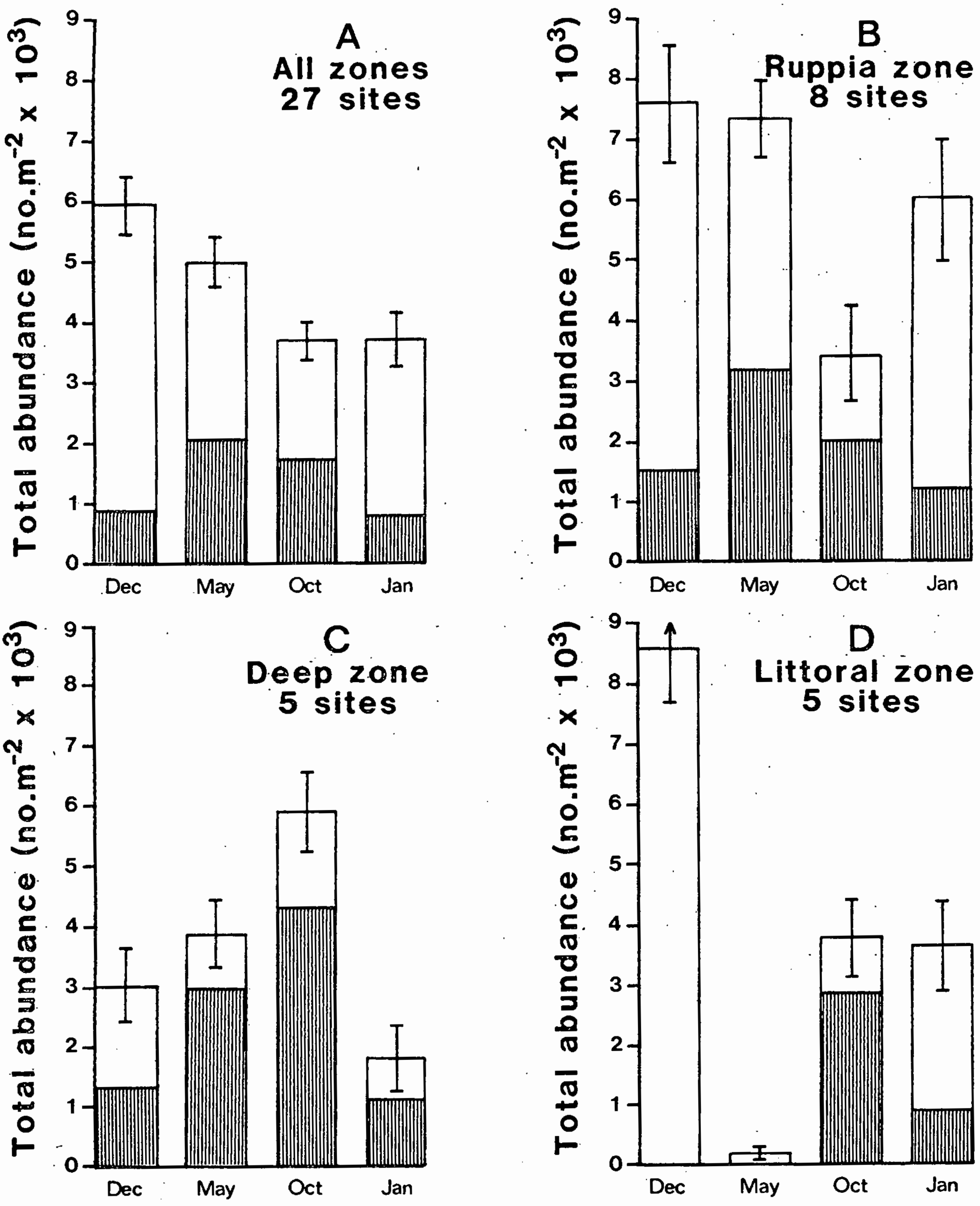

Figure 3: Changes in total abundance (numbers per m^{2}) of the benthic macrofauna of the Bot River estuary at all the sites and in three separate zones during December 1982, May and October 1983 and January 1984. The relative abundance of Apseudes digitalis, the most abundant species, is shown by shading. Vertical bars indicate one standard error of the mean.

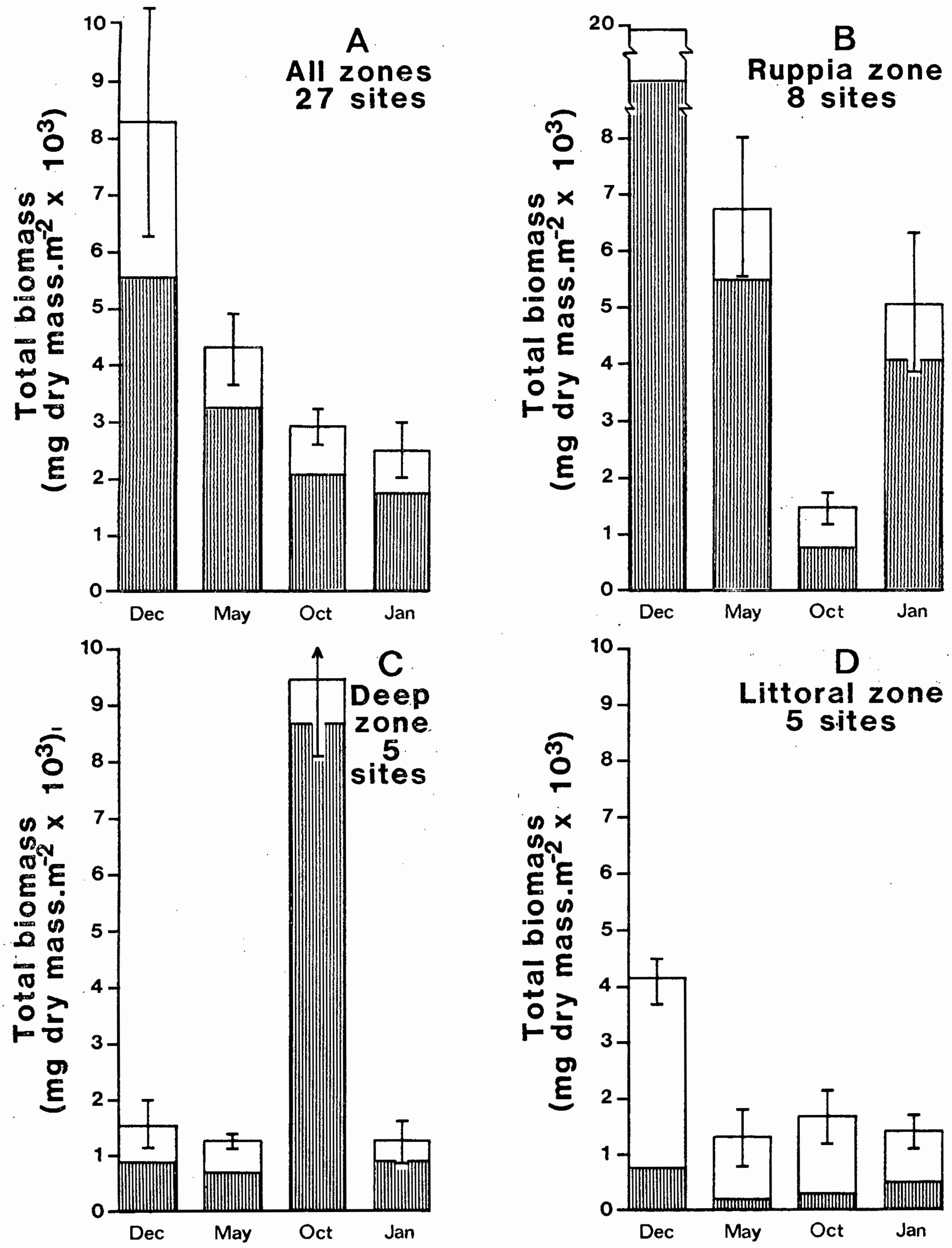

Figure 4: Changes in total biomass (mg dry mass per m^2) of the benthic macrofauna in the Bot River estuary at all the sites and in three separate zones during December 1982, May and October 1983 and January 1984. The relative contribution of <u>Arcuatula capensis</u> is shown by shading. Vertical bars indicate one standard error of the mean.

classification of sites produced by De Decker and Bally (1985).

Abundance and biomass

Figures 3 and 4 illustrate the changes in total abundance and biomass respectively, over all 27 sites and also within each of the three faunal zones separately. Little effect of the breaching in June was discernible in either abundance or biomass levels in October 1983, when all the sites were considered together, although there was a significant reduction of numbers and biomass Figures 3A and 4A). Within each of the individual zones, however, large fluctuations were evident. In the Ruppia zone, a sharp decrease in abundance and biomass occurred in October 1983, with a recovery in January 1984 (Figures 3B and 4B). Almost exactly the reverse was apparent in the Deep zone (Figures 3C and 4C), where numbers and biomass increased in October 1983, returning to more normal levels by January 1984. A large increase in abundance occurred in the Littoral zone after the breaching, but this trend was less clear in the biomass levels (Figures 3D and 4D).

The relative contributions of the single most abundant species, Apseudes digitalis, and the species with the highest biomass, Arcuatula capensis, are superimposed on the histograms in Figures 3 and 4 respectively. Apseudes digitalis represented 35% of the total abundance, on average, while Arcuatula capensis contributed an average of 73% to the total biomass. It is obvious from Figure 3, that total species abundance in the Deep zone was dominated by fluctuations in Apseudes digitalis numbers, while Arcuatula capensis considerably influenced the fluctuations in biomass in all the zones except the Littoral zone, where it forms a

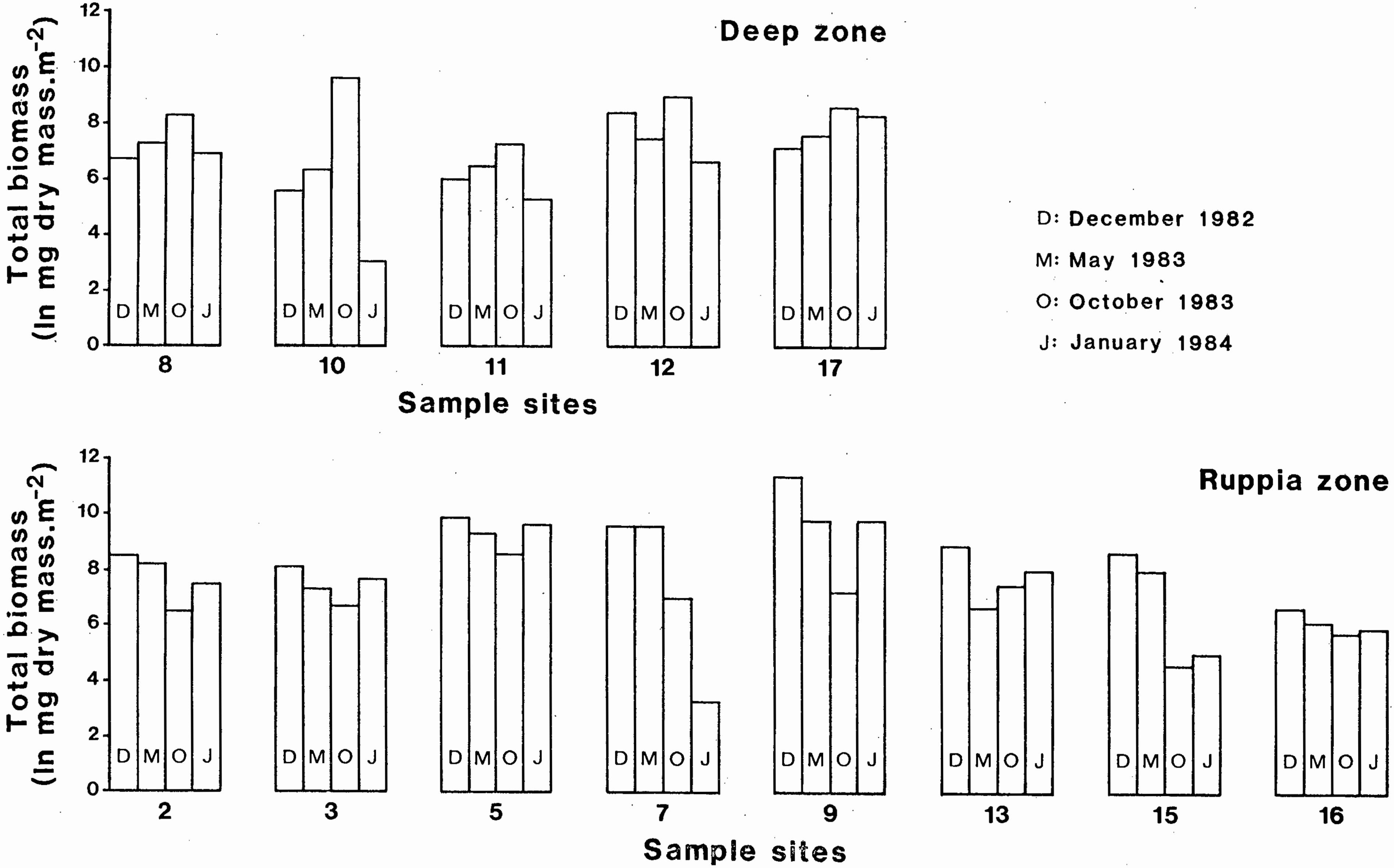

Figure 5: Changes in the total biomass (ln mg dry mass per m^2) at sites of the Deep and *Ruppia* zones in the Bot River estuary during the four survey months.

relatively small component of the fauna (De Decker and Bally 1985). The fluctuations of these and other dominant species, are described in more detail below.

Fluctuations at sites

Between-site comparisons within the zones are useful for determining whether variations were consistent in each zone. Figure 5 shows the fluctuations in biomass at each site representative of the *Ruppia* and Deep zones, since these zones were affected most by the mouth-breaching. Biomass only is illustrated, since abundance revealed similar trends. It is apparent that breaching of the mouth had a consistent effect at almost all sites in both the Deep and *Ruppia* zones, but that the effect was opposite in the two zones. In the Deep zone, an initial slight increase was followed by a sharp increase after the breaching, but levels decreased again by January. An identically opposite trend was evident at almost all of the sites in the *Ruppia* zone: a small decrease in biomass levels from December to May, followed by a sharp decrease in October and a partial recovery at most sites by January 1984. Site 7 in the *Ruppia* zone deviated from this trend because the biomass failed to recover in January, declining even further than before. This can probably be attributed to the complete disappearance of all *Ruppia* from this site after October, making it similar to the sites of the Deep zone, with their reduced abundance and biomass levels.

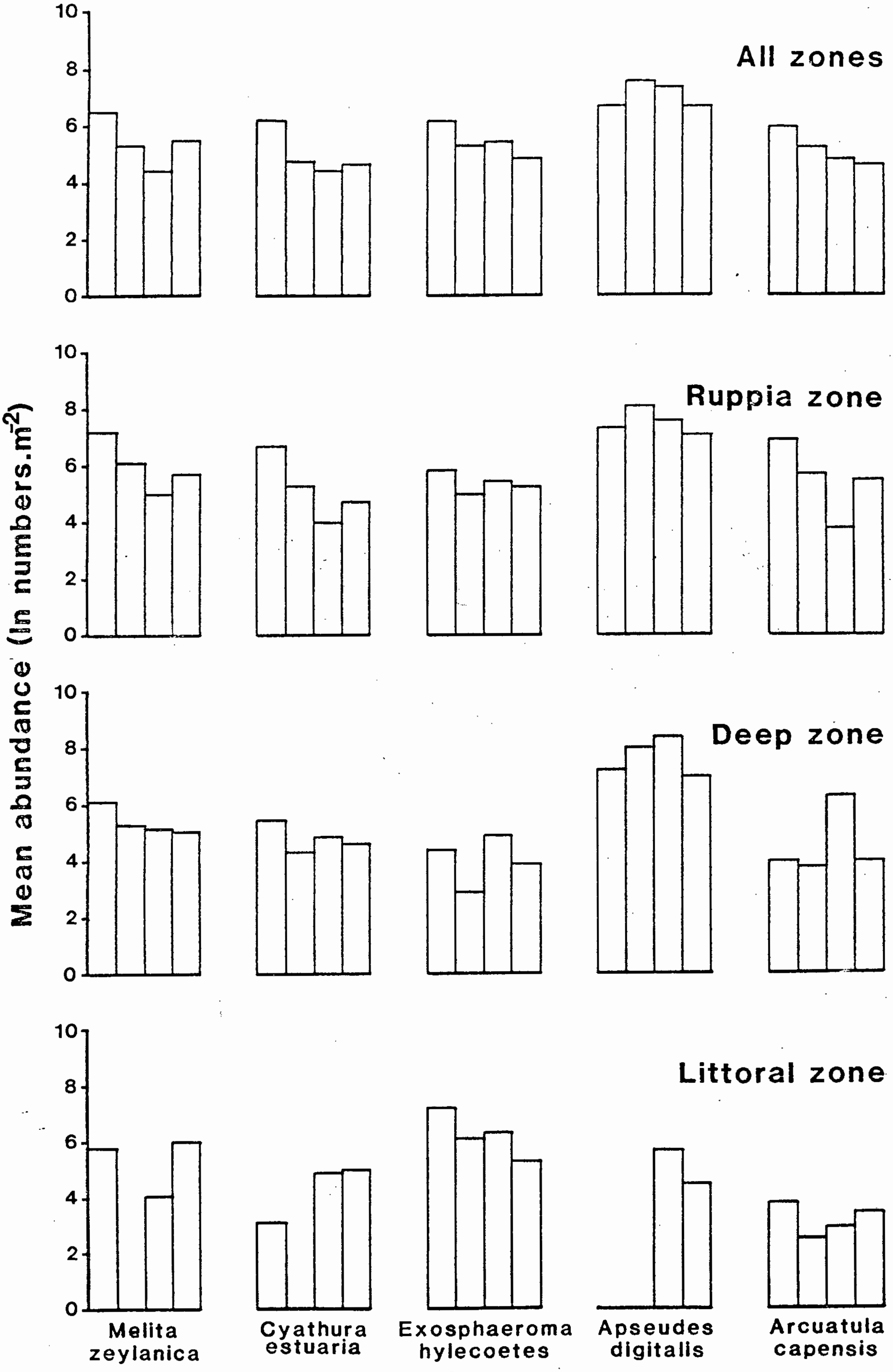

Figure 6: Changes in the mean abundance (ln numbers per m^2) of the five most abundant species in the Bot River estuary in all the zones, and in the three zones separately. The five species together contribute more than 90% to the total standing stock of the benthos.

Fluctuations of individual species

The number of species in the estuary decreased from 23 in May to 15 in October, after breaching of the sandbar (Table 1). By January 1984 this figure had increased only slightly, to 16. It is clear from Table 1 that, of the 9 species which were lost, none contributed more than 5% to the total abundance in May, with the exception of the oligochaetes. A comparison was made of mean abundances for the species *Melita zeylanica*, *Cyathura estuaria*, *Exosphaeroma hylecoetes*, *Apseudes digitalis* and *Arcuatula capensis*, in an attempt to determine their relative contributions to variations in abundance. Figure 6 shows the fluctuations of the species for all sites combined, and in the three zones separately.

In the estuary as a whole, and in the *Ruppia* zone, the mean abundances of all 5 species, excluding *Apseudes digitalis*, showed a downward trend after mouth-breaching, similar to that in Figure 3, suggesting that *Melita zeylanica*, *Cyathura estuaria* and *Arcuatula capensis* were the important species responsible for the large decrease in biomass in October 1983. *Apseudes digitalis* exhibited relatively little change over this period, and showed a similar lack of variation in the *Ruppia* zone, which probably indicates that it was not severely affected by the mouth breaching. The largest decrease in the *Ruppia* zone was presented by *Arcuatula capensis*, while *Exosphaeroma hylecoetes* showed relatively little change due to the breaching and its numbers had, in fact, increased somewhat in this zone by October 1983.

The same combination of species did not, however, contribute to the corresponding increase in abundance in the Deep zone.

TABLE 1: Percentage contributions of all species to the total abundance recorded in each of the four survey months. A hyphen denotes values less than 0.1%.

Species	Dec 1982	May 1983	Oct 1983	Jan 1984
Apseudes digitalis	14.6	40.9	46.2	22.0
Capitella capitata	16.2	8.0	7.7	18.6
Prionospio pernana	11.1	5.5	6.9	21.4
Melita zeylanica	11.5	4.1	2.5	6.3
Actiniaria	2.5	9.1	7.9	6.5
Ceratonereis erythraeensis	8.7	3.8	7.4	2.9
Exosphaeroma hylecoetes	8.1	4.3	6.8	3.6
Arcuatula capensis	6.4	4.0	3.4	2.9
Cyathura estuaria	8.2	2.4	2.5	2.9
Corophium triaenonyx	7.4	0.9	5.5	2.6
Hydrobia sp.		10.5	2.8	4.7
Callianassa kraussi	1.1	0.1	0.3	3.0
Lumbrineris tetraura	0.1	-	-	1.7
Desdemona ornata	0.8	0.1	0.1	
Oligochaeta	0.2	5.6		
Nematoda	1.9			0.5
Chironomid larva	0.9	0.1		
Panathura sp.		0.4	-	0.5
Dipteran larva sp.1	0.1	0.2		
Turbellaria	0.4			
Tomichia sp.		0.1		
Orchestia ancheidos		0.1		
Synidotea sp.		-		-
Pulmonate sp.1		-		
Dipteran larva sp.2		-		
Total number of species	18	23	15	16

Exosphaeroma hylecoetes and Apseudes digitalis reached peak abundances in October, while Cyathuria esturia also increased slightly. It is Arcuatula capensis, however, which made the largest contribution to the increase in both abundance and biomass in this zone after the mouth was opened, offsetting its decrease in importance in the Ruppia zone. This explains the relatively small discernible reduction in overall numbers and biomass of the bivalve in October, when averaged over all sites in the estuary.

In the Littoral zone, the effect of mouth-breaching was negligible. Exosphaeroma hylecoetes is the dominant species here, but its abundance and biomass appear to have been unaffected. An interesting feature of the abundance patterns of Exosphaeroma hylecoetes is that it showed the same variation in all three zones as well as in the estuary as a whole, decreasing in abundance between December 1982 and May 1983, increasing in October after the breaching, and finally decreasing again in January 1984.

Changes in zonation

The changes described above reflect only the responses of individual species to the opening of the estuary. Possible effects on the structure of the communities within each zone now need consideration. A comparison of Figures 7 and 8 reveals the changes which occurred in the spatial pattern of community structure between May and October 1983.

Figure 7, based on samples taken prior to the breaching, indicates the four original faunal zones described by De Decker and Bally (1985): the Deep, Ruppia, Littoral and Sand zones are clearly represented in the dendrogram (Fig. 7A) by distinct clusters of

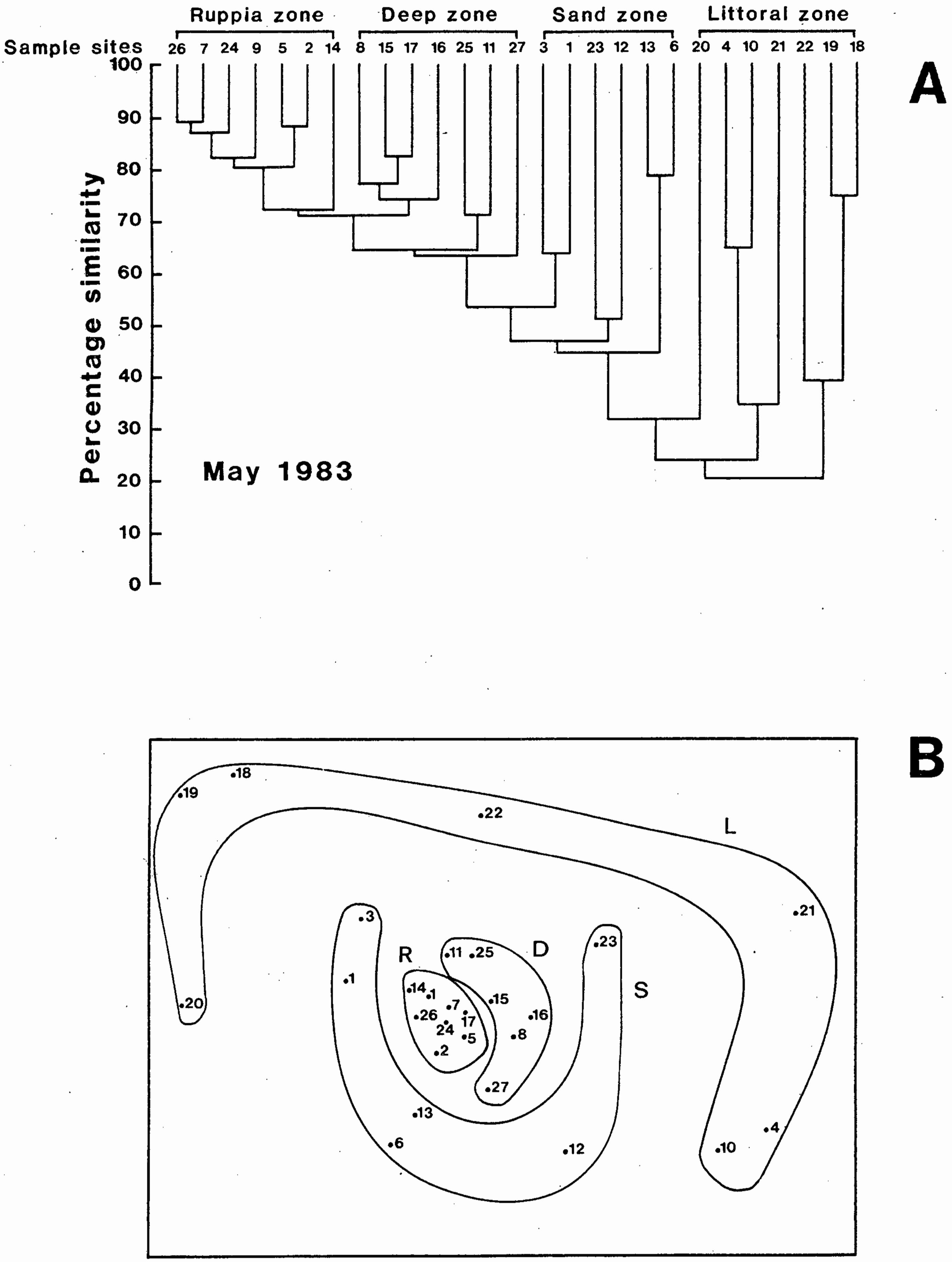

Figure 7: Dendrogram (A) and ordination plot (B) showing the similarities between the sample sites in the Bot River estuary, based on the abundances of benthic species in May 1983, before the mouth was breached. Numbering refers to the sites shown in Figure 1A.

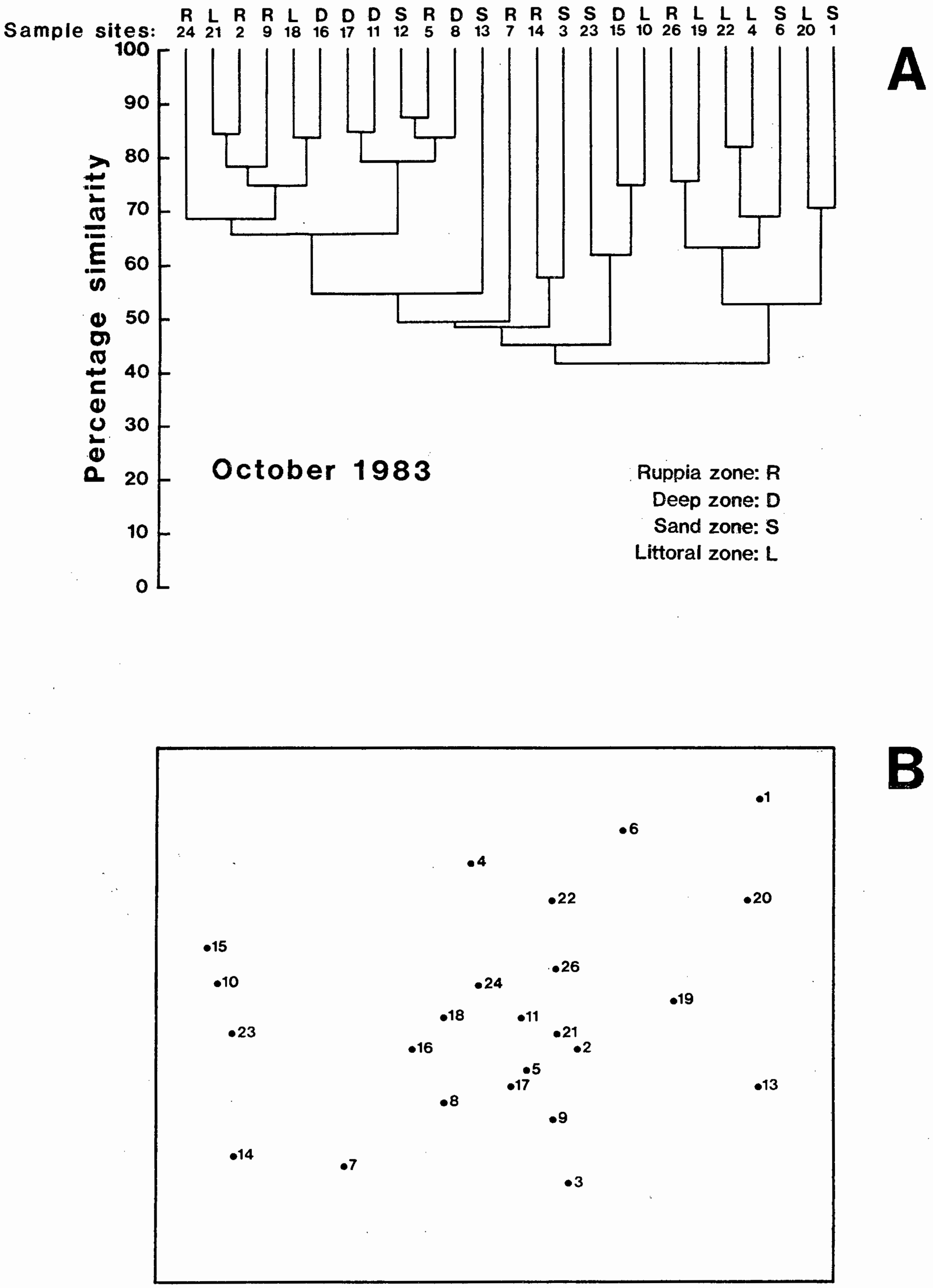

Figure 8: Dendrogram (A) and ordination plot (B) showing the similarities between the sample sites in the Bot River estuary, based on the abundances of benthic species in October 1983, two months after the mouth was breached. Numbering refers to the sites shown in Figure 1A.

sites. These groups are also well-defined in the ordination plot (Fig. 7B), which indicates the relationships between individual sites in each cluster. After mouth-breaching, although some clusters of sites are evident, none of the faunal zones are clearly represented (Fig. 8A). Similarly, no distinct groups are discernible in the ordination plot (Fig. 8B).

De Decker and Bally (1985) have shown that a combined classification of sites sampled during December 1982 and May 1983 produces the original zonation pattern (Fig. 9), with clusters containing sites from both months. This indicates that spatial differences in community structure have greater magnitude than seasonal variations, and suggests that the spatial distribution of species in the Bot River estuary tends to remain constant with time if the estuary is left undisturbed.

The results of a similar combined analysis of species abundances for the May and October 1983 surveys (before and after the breaching), are given by the dendrogram in Figure 10. In this case, the sample sites are tightly clustered into groups which reflect the sampling month, rather than spatial patterns, and some of these represent almost identically the groups defined separately in Figures 7 and 8. This emphasizes the existence of a new spatial pattern of species distribution in the estuarine benthos after the opening of the mouth. Inspection of Figure 10 reveals that groups A to D identify closely with the original faunal zones in Figure 7, while some of the new clusters in Figure 8 can be 'matched' in a similar way. This is clear evidence of the severe disturbance that breaching of the mouth causes to the normally stable pattern of community structure in the benthic macrofauna of the Bot River estuary.

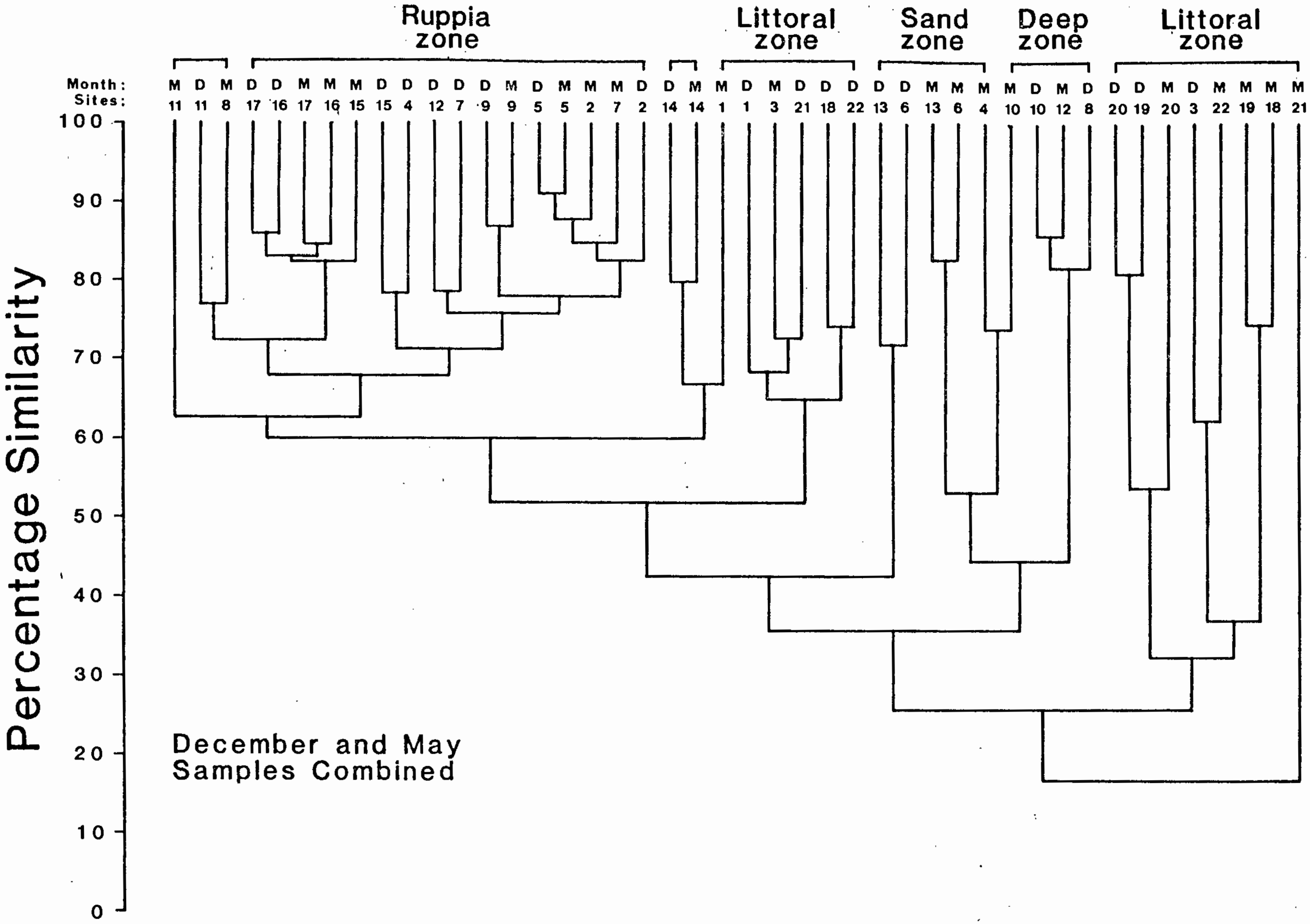

Figure 9: Dendrogram showing the similarities between sample sites in the Bot River estuary based on the abundances of benthic species in December 1982 (D) and May 1983 (M), combined. Numbering refers to the sites shown in Figure 1A (reproduced from Decker & Bally 1985).

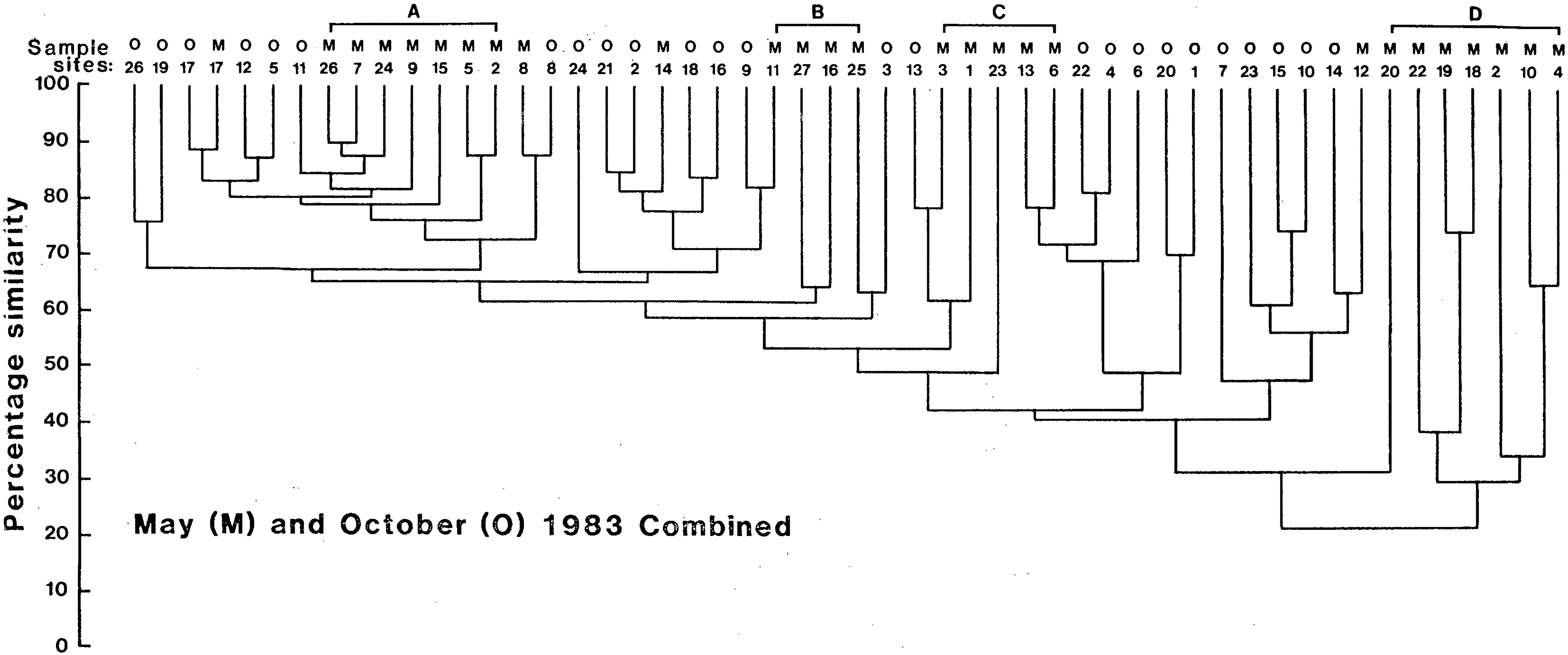

Figure 10: Dendrogram showing the similarities between sample sites in the Bot River estuary, based on the abundances of benthic species in May (M) and October 1983 (O), combined. Numbering refers to the sites shown in Figure 1A. Clusters A, B, C and D correspond to the four clusters, Ruppia, Deep, Sand and Littoral in Figure 7.

DISCUSSION

Breaching of the sandbar between the Bot River estuary and the sea has both an immediate impact on the resident benthic invertebrate populations, as well as a longer-term effect which, taken in consideration with a history of unpredictable breachings (Bally 1985), has a bearing on species composition and diversity. The immediate physical changes following the breaching are the stranding of almost 60% of the macrophytes and the mortality of the remaining submerged plants, due to their intolerance to the salinity levels of seawater. A further important change is the replacement of the wind-generated current regime, which has maximum velocities along the shores (Van Foreest 1985), by a tidally-dominated pattern producing the highest velocities in the deeper, central areas, especially near the mouth.

The short-term effect is particularly evident in the large decrease in faunal abundance and biomass of the communities in the Ruppia zone. This is exemplified by the reduced densities of three dominant species - Melita zeylanica, Cyathura estuaria and Arcuatula capensis - which are all typical of this zone (De Decker & Bally 1985). In the case of the last species, a bivalve, the drop in numbers can probably be attributed to a reduction in available sites for attachment, since the roots of submerged macrophytes provide a substrate for estuarine bivalves in soft sediments (Davies 1982). For several weeks after the destruction of the Ruppia colonies by increased salinities, large numbers of dead Arcuatula capensis were washed ashore (pers. obs.). A similar decrease in standing stocks of the bivalve Musculus virgiliae was recorded by Davies (1982), after the collapse of

Potamogeton pectinatus communities in Swartvlei, a coastal lake in the southern Cape.

Another reason for the reduction of standing stocks in this zone could be an increase in predation after the loss of macrophytic cover. In a study of macrofaunal densities in a seagrass community, Homziak *et al*. (1982) demonstrated an exponential increase in species abundance and diversity with increased macrophytic cover. Peterson (1979) summarised 11 studies showing increases in both faunal densities and species richness after the exclusion of predators, which was partially achieved by denser macrophytic cover. Young *et al*. (1976) similarly found that variations in the intensity of predation could account for differences in species abundance between seagrass-colonised and exposed areas.

A comparison of Figures 7 and 8 shows that not only were densities reduced in the *Ruppia* zone, but the faunal zonation was destroyed as well. It will be shown (Chapter Three) that the spatial distributions of *Ruppia* and of sandy sediments are the two most important factors structuring invertebrate communities in the estuary. It therefore follows that a collapse of the macrophyte will lead to the destruction of the faunal community associated with it.

In the Deep zone, on the other hand, the densities of four of the five dominant species increased. An interesting feature was the increase of the filter feeder, *Arcuatula capensis*, despite the absence of macrophytes to provide a substratum. No comparable increase occurred in the macrophyte-free areas of Swartvlei after the collapse of the macrophytes there. The newly-active tidal currents in the open Bot River estuary may have played a role in this increase, by transporting debris from the decaying macrophytes in the shallows to the faunal communities in the deeper regions. It must be noted that not only *Arcuatula capensis*, but almost all of the dominant species in the Deep zone benefitted in a similar way from this presumed increase in nutrients in the deeper regions (Fig. 6). By January 1984 abundances and biomasses had returned to their usual low levels in the Deep zone and this can conversely be attributed to the cessation of tidal currents after mouth closure.

One of the consequences of having extended periods when the estuary is closed, and brief tidal periods of three to four months following a breaching of the mouth (Bally 1985), is the extremely low species diversity in the Bot River estuary (De Decker & Bally 1985). Low diversity is typical of closed estuaries, resulting from the limited recruitment of new species from the sea (Day 1964). Separation from the sea therefore prevents the estuary from becoming faunistically as diverse as open estuaries, which typically have many more species (Day 1981). By comparing the faunal composition of the estuary to that of coastal lakes (Allanson 1981, Davies 1984), and in particular to the relict estuarine fauna of Lake Sibaya (Allanson *et al*. 1966), De Decker and Bally (1985) have concluded that the Bot River estuary would probably develop into a coastal lake, if the mouth were left intact. This development is interrupted, however, each time the sandbar is breached, as shown by the complete loss of 8 species from May to October (Table 1). Diversity is therefore even lower than that recorded in most closed estuaries (Day 1964, 1981).

Interestingly, no species which contributed more than 5% to the total abundance in May (except for an oligochaete), were absent in

October, following breaching of the mouth. The same persistence of 8 dominant species in the face of annual catastrophic disturbance by extremely low oxygen levels, was noted by Santos and Simon (1980) in Tampa Bay estuary. This probably indicates a difference in niche dimensions: the dominant species represent a group of resilient "estuarine" species, able to survive a wide range of changing environmental conditions, while the eliminated set of species possibly represents part of an emerging "lagoonal" fauna, with a much narrower tolerance to fluctuating conditions.

Zajac and Whitlatch (1982) have suggested, on the basis of a study in a small estuary in southern Connecticut, that the recolonisation of species after a disturbance depends on the nature of the changes in the environment, patterns of resource utilisation and the life histories of colonising species. During the closed period, once riverine input reduces salinities, the physical conditions in the estuary change to those of a coastal lagoon (Allanson 1981), and the development of a fauna similar to that of a coastal lagoon is therefore not surprising. Repeated and unpredictable mouth-breachings, however, interrupt this development by catastrophic disturbances, producing an unstable system with a very low species diversity (Fishelson 1977, Huston 1979).

The periodic opening of the Bot River estuary to the sea may therefore temporarily alleviate the problems of high water levels and low salinities, but it leaves the benthic macrofauna of the system in an ecological limbo, somewhere between an estuary and a coastal lake.

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CHAPTER THREE

An investigation of the sedimentary factors structuring benthic macrofaunal communities in the Bot River estuary, South Africa, using multiple discriminant analysis

ABSTRACT

The structure of estuarine benthic macrofaunal communities is typically strongly influenced by variations in physical and hydrological factors, particularly salinity, temperature and dissolved oxygen. The Bot River estuary, however, being normally closed to the sea and subjected to strong wind-mixing, is vertically and horizontally homogeneous with respect to these factors. Three distinct communities can nevertheless be identified within the benthic macrofauna, which appear to conform fairly well to known patterns of sediment distribution in the estuary. A relationship between sediment-type and community structure is further indicated by numerical classifications of the sample sites in terms of their species composition and sediment characteristics, which independently produce strikingly similar clusters. In this study, multiple discriminant analysis is used to show that a zonal pattern of community structure exists in the benthic macrofauna, determined by corresponding spatial variations in the proportions of medium sand and *Ruppia* debris in the sediments, the latter characteristic indicating the density of colonisation by *Ruppia maritima*.

INTRODUCTION

In their study of the benthic macrofauna of the Bot River estuary, De Decker and Bally (1985) were able to define three distinct faunal communities on the basis of their species composition. These corresponded roughly with three different spatial zones in the estuary (Ruppia, Littoral and Deep zones), identified by the presence or absence of the macrophyte Ruppia maritima and by the relative proportion of sand in the substrata (Willis 1985). The objective here is to provide quantitative evidence to substantiate the apparent correlation between sediment-type and species composition noted by De Decker and Bally (1985).

A method for identifying visually, although without statistical rigour, the important environmental factors which distinguish between clusters of sample sites of similar species composition, has been suggested by Field et al. (1982). By representing each variable quantitatively at each site on a two-dimensional ordination of the groups in the species-space, the environmental variables which differ most between the clusters can be determined. This method has the practical limitations, however, of requiring clear separation of groups and, more importantly, a consideration of the effects of each variable in isolation. As distribution patterns of species are likely to be determined simultaneously by many interacting environmental influences, an investigation of their combined effects often makes greater ecological sense. This can be seen as an extension of the multi-dimensional niche-space of interacting environmental variables proposed by Hutchinson (1957).

Application of the multivariate technique of multiple discriminant analysis (MDA) to the problem of identifying the environmental factors determining the distribution of species in aquatic benthic communities, is suggested by Green and Vascotto (1978). This technique enables one to define quantitatively the relative importance of individual environmental variables to the differentiation of clusters, by maximising the ratio of between- to within-group variances for the sites, within the multi-dimensional space defined by the complete set of variables simultaneously. The data set can thus be reduced to contain only those variables which discriminate maximally between the clusters. In this way, Green and Vascotto (1978) were able to identify, from 11 environmental variables, an increasing linear trend in the depths and surface areas of lakes having good wind-driven circulation, which represents the continuum from eutrophy to oligotrophy. Shin (1982) has used MDA to relate differences in the species composition of benthic communities in North Bay, western Ireland, to the relative organic carbon content and fine silt-clay fraction of the sediment.

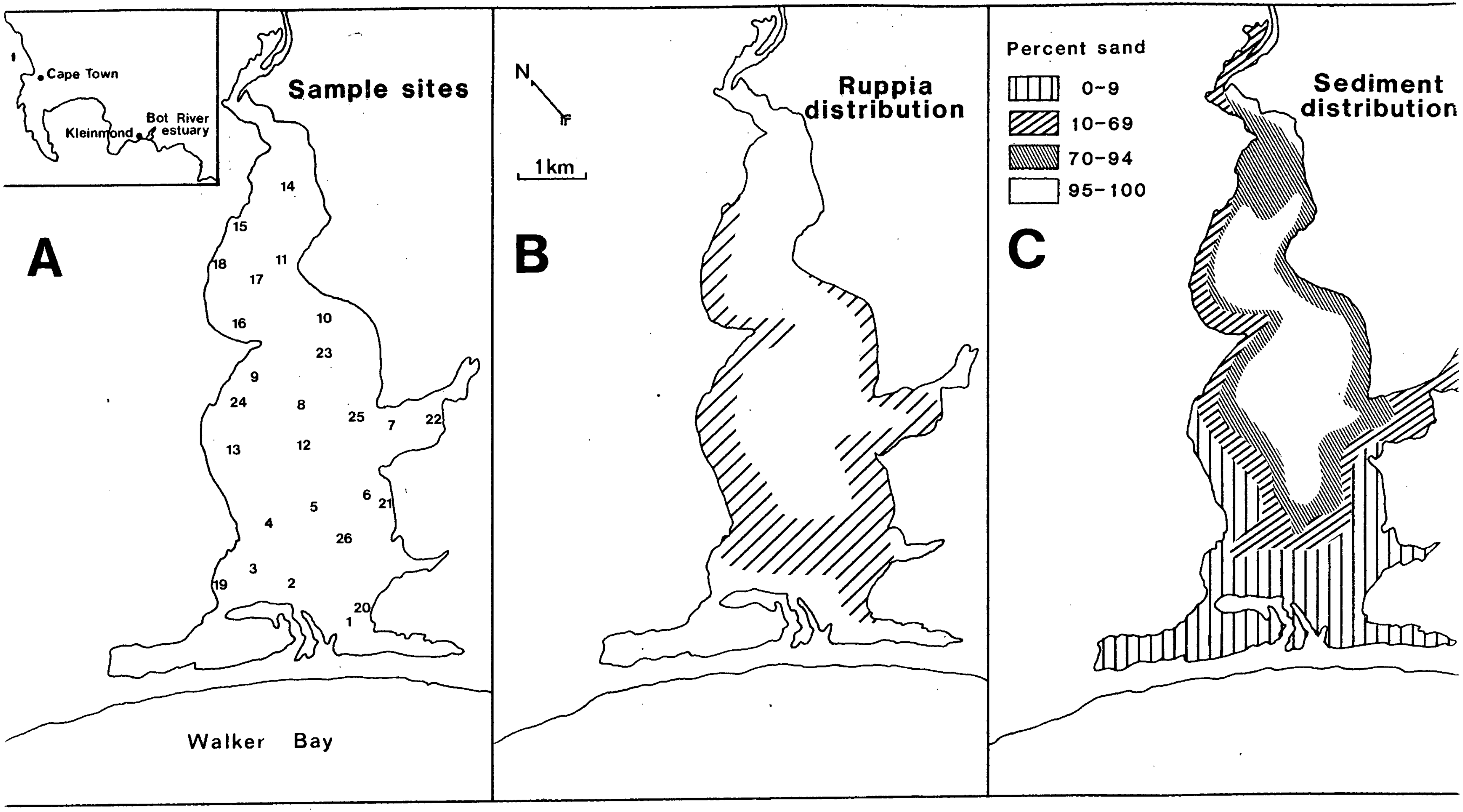


Figure 1: Map of the Bot River estuary showing sample sites, *Ruppia* distribution and sediment distribution. (Sedimentology after Willis (1985)).

MATERIALS AND METHODS

Study site

A comprehensive description of the environmental parameters and sediment distribution within the estuary has been given by Willis (1985). However, a brief summary of the details relevant to this study follows here.

The estuary is not normally tidal, being separated from the sea by a sandbar, and therefore is not characterised by the strong vertical and horizontal salinity stratification typical of many open estuaries (Day 1981). Vertical mixing of the water in the shallow basin (maximum depth of 5m) is complete throughout the year, due to the frequent occurrence of strong winds. As a result, salinities, for example, varied horizontally only between 36‰ and 40‰ over all sites during the survey, with no vertical stratification.

A wide band of Ruppia maritima colonises the fringe of the estuary, leaving the central area clear of macrophytes (Fig. 1B). Sandy sediments occur mainly along the shores of the southern parts, where wave action removes the lighter sediment fractions, while mud is found in the deeper central and northern areas (Fig. 1C).

Collection of samples

Samples of benthic fauna and sediments were collected from 24 sites in the Bot River estuary (Fig. 1A) during April 1984. A box corer (15cm x 11cm x 9cm; 165cm^2 cutting area) was used to collect duplicate samples of benthic fauna at each site, and its application and limitations have been described by De Decker and Bally (1985). Sites 14 and 24 were omitted in this survey, due to complete exposure (Site 14) or unreliability of the data (Site 24).

Sediment samples were taken simultaneously at the same 24 sites, with a 35cm long hand-corer made of PVC piping of 42mm diameter. This was plunged to a depth of 20cm, and a rubber stopper inserted into its upper end to enable withdrawal of the sediment core. The procedure was repeated if any sediment was lost. The cores were extruded into plastic bags and frozen within 12 hours of collection. Sediments were analysed by the methods reported in Willis (1985). Particle size analysis was carried out by wet-sieving the cores through 2mm and 63 micron sieves to separate the gravel and mud fractions from the sand fraction, which was then further analysed into size fractions (very coarse, coarse, medium, fine and very fine) with a settling column, using 3 to 4g subsamples. Organic matter was determined by calculation from loss of weight at ignition (1050 °C).

In previous surveys, good correlations have been found between the amount of *Ruppia* debris in the faunal sample cores and the density of living *Ruppia* at the sample sites (pers. obs.). The debris in the cores represents root material as well as dead *Ruppia* plants and, since it has a direct influence on the environment of a benthic organism, the material was separated from the box cores, dried for 24 hours in an oven at 60 °C and weighed. These data (in g dry weight per m^2) were used to represent the contribution of *Ruppia* debris to the sediment composition of each sample site, and complete the set of nine sediment variables - gravel, very coarse sand, coarse sand, medium sand, fine sand, very fine sand, mud, organic matter and *Ruppia* debris.

Data analyses

Cluster analysis

The raw species abundances were transformed to log(Xij+1) and the sediment data to arcsin(Xij), where Xij is the numerical value of the ith variable at the jth site, in order to reduce the effect of very large values on the cluster analyses (Zar 1974, Clifford & Stephenson 1975).

A numerical classification of the sites was performed for each data set, using the Bray-Curtis similarity coefficient (Bray & Curtis 1957) and the method of group-average sorting (Clifford & Stephenson 1975, Field *et al*. 1982) for clustering the sample sites, to produce dendrograms defining groups of sites at different levels of similarity. Relationships between individual sites within the hyperspace defined by each set of variables, are also illustrated by distances on ordination plots. These are produced by non-metric multi-dimensional scaling of the similarity matrix (Kruskal & Wish 1978), and represent the projection of the site configurations onto a plane in a manner which attempts to preserve as much as possible of the information in the original variable-space.

Information statistic tests

The species composition in both sets of site groups was investigated, using information statistic tests for pair-wise comparison of groups on presence-absence of species. Although lacking in statistical rigour, the calculated statistic ($2\Delta I$) has an approximate chi-square distribution and can be used to indicate the species which differ most between any two groups of sites (Field 1969, Field *et al*. 1982).

Multiple discriminant analysis

A stepwise MDA of the sediment variables was performed, using the BMDP statistical package, P7M (Jennrich & Sampson 1983), to determine the sediment factors which describe most of the variation in species composition between the clusters, as identified by their species abundances (site-by-species groups). Although MDA is based on the assumptions of multinormality and linearity of the data, it is fairly robust (Pimentel 1979). Linearising transformations such as those used, tend to improve the approximation to these conditions (Zar 1974); gravel was nevertheless excluded from the sediment data for this analysis, as it showed marked skewness in its distribution. In addition, all data were ignored for Site 1 (Fig. 1), which was identified as an outlier, being uniquely situated in the channel scoured by tidal currents after breaching of the mouth took place in June 1983. The further condition of homogeneity of within-group variances was satisfied by standardisation of the variables to distances from a mean of zero,in units of their standard deviation. All computing was done on the Sperry 1100 computer at the University of Cape Town.

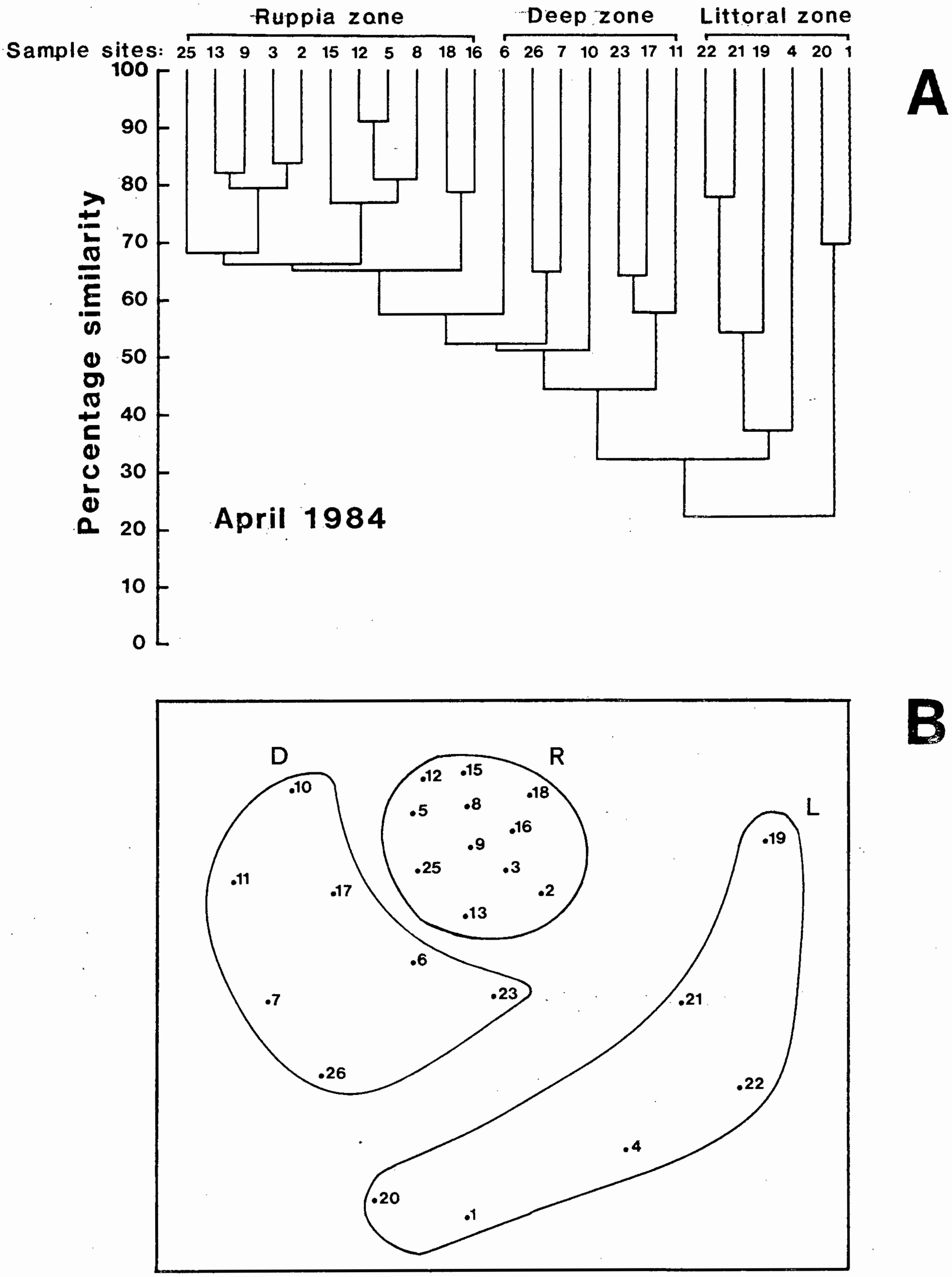


Figure 2: Dendrogram (A) and ordination plot (B) showing similarities between the sample sites in the Bot River estuary, based on species abundances recorded in April 1984. Numbering refers to the sites shown in Figure 1A.

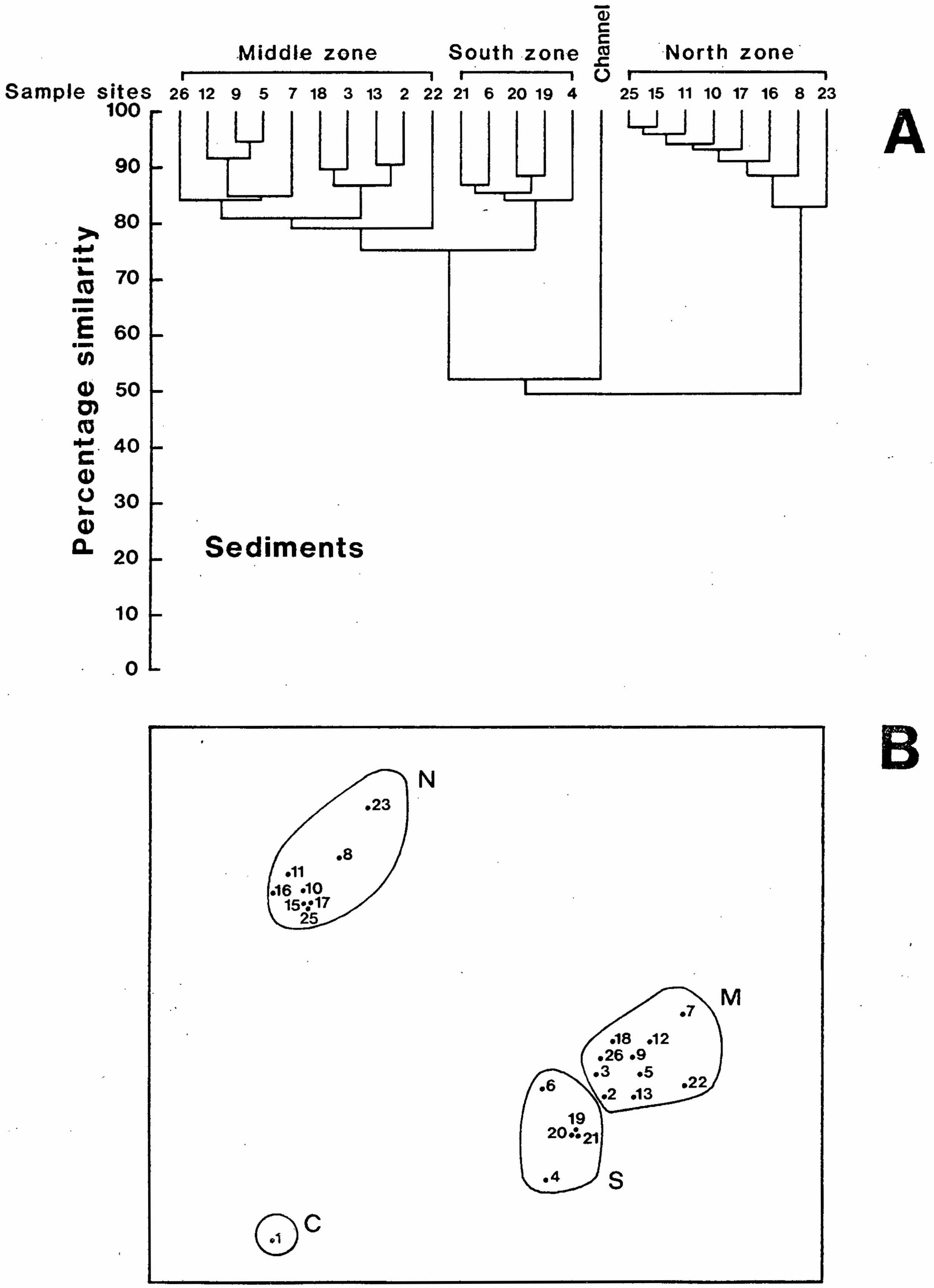


Figure 3: Dendrogram (A) and ordination plot (B) showing similarities between the sample sites in the Bot River estuary, based on an analysis of sediments, including <u>Ruppia</u> debris, at each site. Numbering refers to the sites shown in Figure 1A.

RESULTS

Sixteen species were recorded in this survey, of which three were excluded from the present analysis, as they appeared at fewer than three sites. The remaining 13 (Table 1) together contribute more than 93% to the total abundance recorded, and were therefore considered to be sufficiently representative of the species composition of the estuary (De Decker & Bally 1985). The complete set of sediment variables is given in Table 2.

Cluster analysis

Figures 2 and 3 show the dendrograms and corresponding two-dimensional ordinations of sites, based on their species abundances and sediment characteristics respectively. Three distinct clusters of sites can be identified in both Figures 2 and 3, and a striking similarity between the two configurations is apparent. The groupings obtained in Figure 2 correspond well with those found by De Decker and Bally (1985), and are therefore identified by the same names: Ruppia (R), Littoral (L) and Deep (D). The sites in the Ruppia group, at more than 60% similarity, form a tight cluster, differentiated at similarity levels of between 60% and 40% from sites in the Deep group, while the Littoral group is less than 40% similar to both of these clusters.

In Figure 3, the three sediment clusters (North (N), Middle (M) and South (S) zones) are distinguishable at a similarity level of 76%. Site 1, in the channel (Fig. 1A), is very dissimilar to all the other sites, having an extremely high proportion of very coarse and coarse sand in its sediments (Table 2).

Table 1: Comparisons of the species compositions of the *Ruppia* (R) and Littoral (L) groups, and the Middle (M) and South (S) zones. Significance levels (p) are indicated by *=5%, **=1%.

Species	R	L	p	M	S	p
Corophium triaenonyx	11	1	**	9	2	*
Actiniaria	11	0	**	9	1	**
Arcuatula capensis	10	0	**	8	1	*
Prionospio pernana	8	0	**	7	0	**
Cyathura estuaria	11	3	*	8	3	
Capitella capitata	8	1	*	6	2	
Panathura	7	1		6	1	
Melita zeylanica	6	3		5	2	
Exosphaeroma hylecoetes	6	3		5	3	
Hydrobia	5	5		7	3	
Ceratonereis erythraensis	4	4		3	3	
Scoloplos uniramus	3	2		3	1	

Table 2: Sediment data obtained at 24 sites in the Bot River estuary during April 1984 (GRAV=gravel, VCS=very coarse sand, CS=coarse sand, MS=medium sand, FS=fine sand, VFS=very fine sand, MUD=mud, ORGM=organic matter, RDEB=*Ruppia* debris).

Site	GRAV	VCS	CS	MS	FS	VFS	MUD	ORGM	RDEB
01	0.7	8.1	75.9	15.0	0.4	0.0	0.0	0.00	1.44
02	0.1	0.7	15.7	33.1	43.2	0.2	7.1	0.85	13.40
03	0.6	0.0	8.1	71.5	16.2	1.6	2.0	1.73	13.85
04	0.0	0.0	6.0	74.5	19.5	0.0	0.0	0.25	4.81
05	0.0	0.0	2.2	36.4	41.7	13.1	6.6	5.27	3.11
06	0.2	0.0	4.1	72.9	14.3	0.0	8.4	0.59	0.41
07	0.9	0.0	0.5	24.4	60.3	9.4	4.5	3.73	0.00
08	0.0	0.0	0.0	1.8	0.0	0.0	98.2	3.80	2.41
09	0.0	0.0	0.9	30.1	38.0	19.5	11.5	3.58	7.89
10	0.8	0.0	0.0	12.8	0.0	0.0	86.4	4.05	0.59
11	1.5	0.0	0.0	2.4	0.0	0.0	96.1	4.01	0.44
12	0.0	0.0	0.1	24.6	34.6	27.0	13.7	4.25	0.26
13	0.0	0.5	2.1	53.8	38.9	0.5	4.2	1.40	8.96
15	1.0	0.0	0.0	5.0	0.0	0.0	94.0	3.47	1.19
16	0.5	0.0	0.0	0.7	0.0	0.0	98.8	3.37	5.63
17	4.1	0.0	0.0	7.4	0.0	0.0	88.5	3.42	4.00
18	1.7	0.1	10.3	41.5	28.8	7.7	9.9	1.91	15.99
19	0.0	0.0	6.6	86.3	5.0	0.0	2.1	0.41	9.11
20	0.0	0.0	15.4	77.5	6.1	0.0	1.1	5.98	0.63
21	0.0	0.0	1.4	63.1	33.3	0.0	2.3	0.16	1.33
22	0.0	0.0	1.4	49.9	41.6	0.2	6.9	0.29	0.00
23	0.0	0.0	0.0	4.6	0.0	0.0	95.4	3.33	0.00
25	1.5	0.0	0.0	4.8	0.0	0.0	93.8	4.61	3.70
26	0.3	0.0	4.2	53.1	36.7	5.4	0.3	4.48	0.85

Figure 4 shows that these three sediment clusters correspond closely, in their spatial configuration, to the zonal pattern of sediment distribution given in Figure 1C. Misclassified sites tend to be situated along the boundaries of zones, where sediment characteristics can be expected to be transitional. By plotting the site-by-species groups (R, L and D) onto the sediment zonation shown in Figure 4, the correspondence between the classifications of sites by their species abundances and by their sediment characteristics is clearly evident.

Information statistic tests

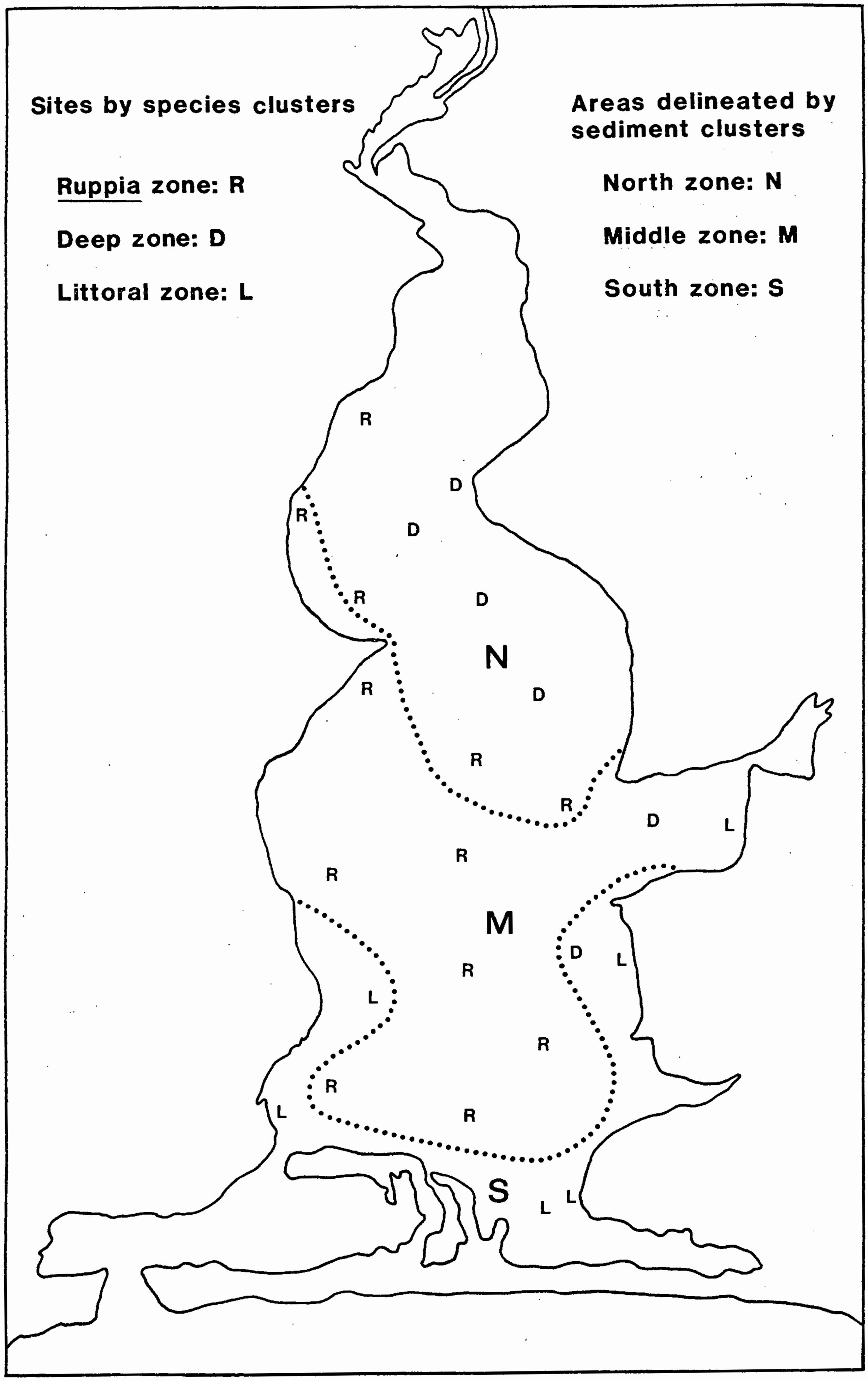
The results of the information statistic tests for differences in species composition between groups R and L, and between groups M and S, are given in Table 1. It is clear, by inspection, that the community structure of the corresponding groups (R and M; L and S) is approximately equivalent, with four species in particular - Actiniaria, *Corophium triaenonyx*, *Arcuatula capensis* and *Prionospio pernana* - all of which are common in groups R and M, and absent or rare in groups L and S, characterising the difference in species composition between the two pairs of groups.

Pairwise comparisons of group D with groups R and L, and of group N with groups M and S, yielded similar parallels in species composition, but the results were not "significant", suggesting that groups D and N represent less well-defined communities.

Multiple discriminant analysis

By this technique, the total amount of variation in the sediment data between the three site-by-species clusters (R, L and D) can be visually represented by the site configurations on a single

Figure 4: Comparison of the spatial distributions of the clusters in Figures 2 and 3, as identified by their species and sediment composition, respectively. The sites are labelled according to their group membership in Figure 2 and are separated by the dotted lines into the zones defined by the sediment clusters (M, N and S) in Figure 3.


Sites by species clusters
Ruppia zone: R
Deep zone: D
Littoral zone: L
Areas delineated by sediment clusters
North zone: N
Middle zone: M
South zone: S
N
M
S

plane, defined by two composite variables (discriminant functions) which are linear combinations of all eight original sediment variables. These two axes define the directions of maximum between-groups dispersion, and can be interpreted in terms of the relative contributions of the original variables to each (Legendre & Legendre 1983).

The results are presented in Table 3. The two discriminant functions, DF I and DF II, describe 67% and 33% respectively, of the variation between the clusters. Only two sediment variables - medium sand and Ruppia debris - contribute significantly (and almost equally) to each. "Significance" is defined here by an arbitrary cut-off value for the ratio of between- to within-group variance for the variable, and is taken as 4.0.

Figure 5 gives the configuration of the sample sites in the discriminant plane. Group R, having a high proportion of Ruppia debris compared with medium sand, is effectively distinguished by DF I from group L, which has the opposite characteristics (high sand, low Ruppia), while group D is distinguished by DF II from both these groups by having low relative proportions of both sediment types. These properties correspond well to those expected intuitively in the three sediment zones. The sediment characteristics of group D are less distinct, however, only 57% of its sites being correctly classified, the remaining sites having the sediment characteristics of either R or L. This corroborates the results of the information statistic tests, indicating that group D represents a less well-defined sediment zone.

Table 3: Results of the discriminant analysis of sedimentary characteristics between the three faunal zones (*Ruppia*, Littoral and Deep) in the Bot River estuary.

Discriminant function	1	2
Percent of dispersion	66.9	33.1
Cumulative percent of dispersion	66.9	100.0
Discriminant function coefficients		
Medium sand	-1.02	0.70
Ruppia debris	1.03	0.71

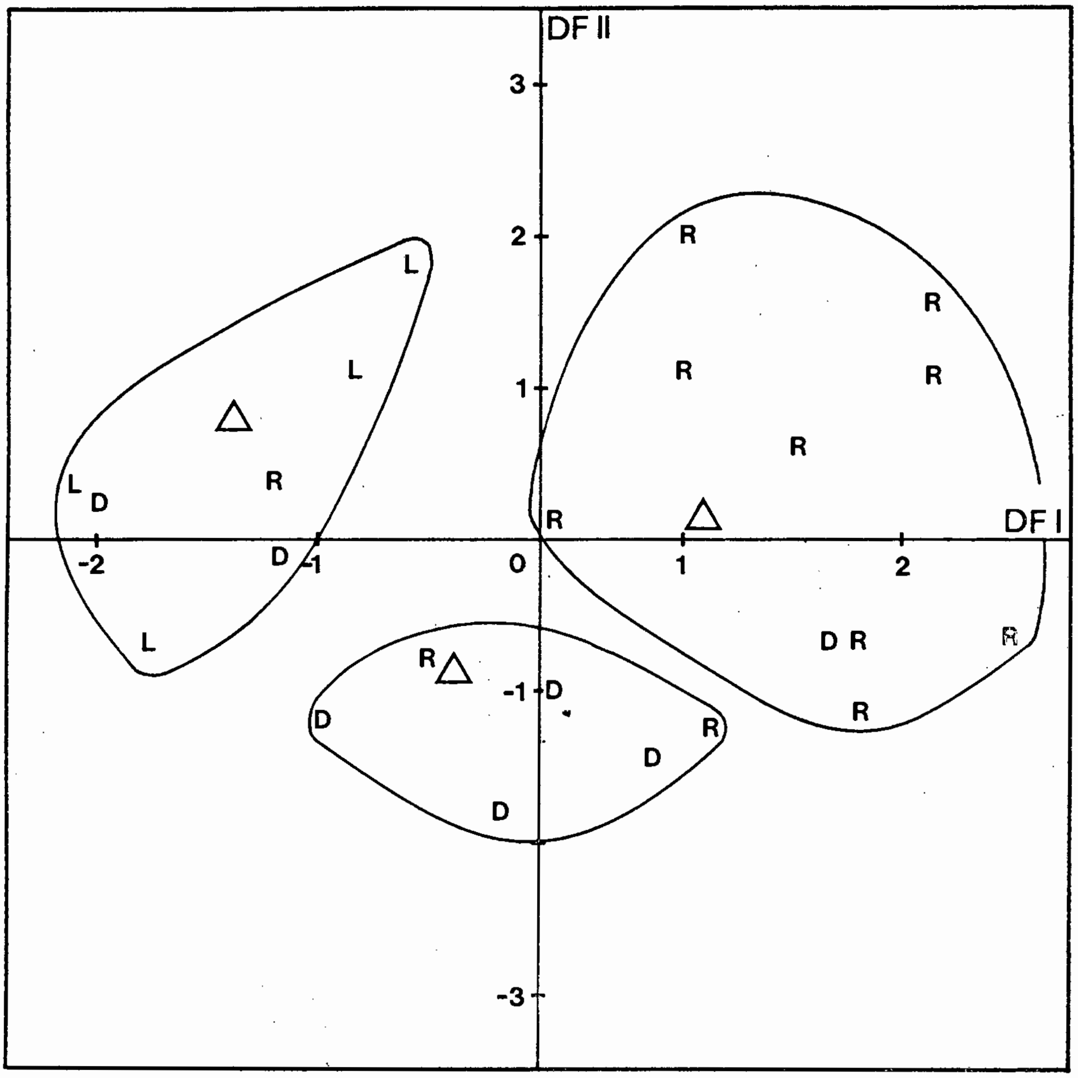


Figure 5: Separation of the site groups *Ruppia* (R), Deep (D) and Littoral (L) on the two discriminant functions (DFI & DFII) of the variables *Ruppia* debris and medium sand. Triangles indicate the position of the centroid of each group.

DISCUSSION

The most important physical factor controlling the distribution of the benthos in open estuaries is usually salinity (Wolff 1973, Day 1981). The benthic fauna of the Knysna estuary, for example, can be divided into five components by their salinity tolerance (Day _et al_. 1952, Day 1967), which are distributed along the horizontal gradient in salinity from freshwater at the head, to seawater at the mouth of the estuary. Ristich _et al_. (1977) have similarly found that salinity is one of the most important determinants of species distributions in the Hudson River estuary. The Bot River estuary, however, is normally cut off from the sea. It therefore lacks the tidal input that causes horizontal salinity variations, and is vertically and horizontally homogeneous with respect to hydrological factors, due to mixing of the water column by frequent, strong winds over the shallow basin. Salinity can therefore have no controlling influence over community boundaries in the Bot River estuary when it is closed.

Cluster analysis of the species abundances (Fig. 2), however, shows that discrete groups of species exist and that their distributions can be identified (Fig. 4). Unlike the widely differing species compositions of separate communities in open estuaries, however, these groups have many species in common, but they are distinguishable by the relative abundances of the dominant species in each. The difficulty experienced in defining the "indicator species" in the Deep zone by information statistic tests, can probably be attributed to the extremely low abundances found in this zone by De Decker and Bally (1985). The low abundances preclude the formation of a distinct community, and could be due to the unsuitability of a muddy substratum for many benthic species, especially filter feeders (Boyden & Little 1973, Mann 1982).

The second-most important physical factor determining community boundaries in open estuaries is often the nature of the substrata. In these estuaries, for example the Tay estuary (Khayrallah 1975) and the Knysna estuary (Day *et al.* 1952), tidal currents remove the fines from the central, deep areas and deposit them in mudflats along the shores. In the Bot River estuary the opposite occurs, because wind-generated waves remove the fines from the sediments along the shores and deposit them in the deeper, central areas (Willis 1985). Only once the sandbar has been breached, is a channel with a coarse substratum scoured in the region of the mouth, where current velocities are highest. This wind-induced sorting therefore produces the sediment distribution shown in Figure 1C.

The results of this study indicate that the sediment distribution, as well as the distribution of *Ruppia* debris, are the most important factors determining community structure in the Bot River estuary. The spatial differentiation of the fauna into the three zones - *Ruppia*, Littoral and Deep - is determined by the two sediment variables, medium sand and *Ruppia* debris, of which the latter is a measure of the density of *Ruppia* colonisation. MDA has thus provided a more precise definition of the sedimentary characteristics of each zone and has shown that they are uniquely differentiated by these two variables predominantly. The results of the information statistic tests suggest strong niche selectivity of the species characteristic of the *Ruppia* zone, which show preference for sediments rich in *Ruppia* debris, while sediments with a high proportion of medium sand, represented by the Littoral zone, support a lower abundance and diversity of species. The Deep zone is less well-defined in terms of its sediment characteristics, and its species composition is correspondingly less distinct.

The techniques used here for investigating the influence of environmental factors on benthic community structure are robust and descriptive in nature, and a quantitative evaluation of group differences has not been attempted. It has been suggested (Green & Vascotto 1978), however, that visual assessment of the "significance" of group differentiation on ecologically interpretable discriminant functions, is possibly a more conservative approach than is the use of formal statistical tests for evaluating group differences in terms of the individual variables (eg. ANOVA or MANOVA). The results instead provide convincing visual evidence of the importance of specific sediment characteristics in determining spatial variations in the macrobenthic communities of the Bot River estuary.

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CHAPTER FOUR

Digestive enzymes
of the crystalline style of
the estuarine bivalve, *Arcuatula capensis* (Krauss):
relation to potential nutritional resources

ABSTRACT

The presence of three carbohydrases (α-amylase, cellulase and laminarinase) and a lytic enzyme in the crystalline style of the estuarine bivalve, Arcuatula capensis, is demonstrated. Enzyme activities of the carbohydrases were determined by incubation with starch, carboxymethylcellulose and laminarin, respectively, followed by measurement of the release of reducing sugars. α-Amylase showed the highest specific activity, and cellulase the lowest. By measuring the lytic activity of an enzyme extract from the style of the bivalve, on four bacterial strains, it is shown that Arcuatula capensis has the ability to digest bacteria, but that the bacterial isolates vary in their sensitivity to the lytic enzymes.

It is assumed that the high α-amylase activity is necessary for digestion by the bivalve of polysaccharides released by bacterial degradation of the refractory debris derived from estuarine macrophytes. The lytic activity of the style enzymes suggests that bacteria play a further important role in providing additional carbon, as well as nitrogen. It is possible that the presence of laminarinase may prove valuable for the digestion of phytoplankton during times when bacterial biomass in the estuary is low, as occurs during periods of very low or high salinities.

INTRODUCTION

Since early work established the association between micro-organisms and particulate organic debris, and the importance of this association as a nutritional resource for macroconsumers (Newell 1965, Darnell 1967, Odum & De la Cruz 1967), the concept of a food chain from debris through micro-organism to macroconsumer, has become entrenched in the literature (Wetzel 1977, Howard-Williams & Davies 1979, Tunnicliffe & Risk 1977, Mann 1982). Recently, however, this sequence has come to be regarded as too simplistic (Christian & Wetzel 1978). Furthermore, it has been shown that marine invertebrates are able to obtain nutritional carbon directly from seaweed debris (Tenore 1981, Stuart _et al_. 1982). Detritus in estuaries, however, is derived from vascular plants, and is more refractory than marine macrophytic debris (Tenore 1981, Findlay & Tenore 1982). Microbial degradation may therefore play a more important role in the supply of nutrients to consumers in estuaries (Findlay & Tenore 1982, Tenore 1983). A fresh approach to problems in nutritional physiology is the analysis of the digestive enzyme systems of invertebrates, in relation to naturally-occurring potential food resources.

The presence of carbohydrases in the crystalline styles of bivalve molluscs has long been recognised (Coupin 1900, Yonge 1923, Lavine 1946). More recently, these enzymes have been characterised (Sova _et al_. 1970, Wojtowicz 1972, Jacober _et al_. 1980) and their relation to the diet of the animals investigated (Crosby & Reid 1971, Kristensen 1972, Gianfreda _et al_. 1979, Seiderer _et al_. 1982). The carbohydrases occur in the form of several iso-enzymes, of which the principal ones are α-amylases (Judd 1979, Seiderer & Newell 1979, Newell *et al.* 1980), cellulases (Yokoe & Yasumasu 1964, Elyakova 1972) and laminarinases (Bull & Chesters 1966, Sova *et al.* 1970). These iso-enzymes digest intracellular polysaccharides (for example, starch), the structural component of cell walls (cellulose) and chrysolaminarin (the major storage product of diatoms (Darley 1977)), respectively. Lytic enzymes in the styles of bivalves have also recently been characterised (McHenery & Birkbeck 1979, McHenery *et al.* 1979) and their importance in the digestion of the bacterial components of the diet has been revealed (McHenery & Birkbeck 1982, Harris 1984, Seiderer *et al.* 1984).

By investigating the presence and activities of carbohydrases and lytic enzymes in a consumer, an assessment of the relative importance of the detrital and microbial components of its diet can be made. In the case of carbohydrases, this has been attempted by relating the levels of reducing sugars released by the style enzymes, to the carbon- and energy-budgets of the animals (Seiderer *et al.* 1982, Harris 1984, Lucas & Newell 1984). It has since been found, however, that large discrepancies exist between the two methods currently used to assay reducing sugars, i.e., the Nelson-Somogyi and 3,5-dinitrosalicylic acid (DNS) methods (Breuil & Saddler 1985, Fielding *et al.* in prep.). In addition, energy-budget calculations such as this are considered by Fielding *et al.* (in prep.) to be incorrect, due to the inappropriate use of a glucose standard. Calculations of this type may therefore be premature while the exact nature of the released sugars remains unknown. It is nevertheless assumed that the presence of suitable enzymes indicates a potential for the utilisation of particular food resources. Crosby and Reid (1971) for example, have found good correlations between cellulase activities and the cellulose content of the food of 9 bivalve species.

Arcuatula capensis (Krauss), an estuarine bivalve, is a suspension feeder associated with the Ruppia maritima beds in the Bot River estuary (De Decker & Bally 1985). Its potential nutritional resources consequently consist of suspended detrital particles, phytoplankton and free-floating bacteria. While suspended detritus is in abundance, at annual mean levels of $16mgl^{-1}$ (Roberts 1984), like most estuarine particulate debris it is highly refractory (Tenore 1981). Chlorophyll levels in the phytoplankton are fairly low, probably due to high turbidity in the estuary, and show little temporal variation, fluctuating between $0.4mgm^{-3}$ and $6.0mgm^{-3}$, about a mean of $1.9mgm^{-3}$(Bally et al. 1985). Levels of $1.0mgm^{-3}$ were measured at the time and locality at which the bivalves were sampled. Average bacterial biomass in the water column, however, is very high due to the abundance of large rods in the estuary (Roberts et al. 1985a), and fluctuates annually between $134mgm^{-3}$ and $295mgm^{-3}$.

The aim of this study is to test for the presence of lytic enzymes and the three carbohydrases - α-amylase, cellulase and laminarinase - in the crystalline style of Arcuatula capensis, and to relate their activities to the nutritional resources available to the bivalve.

MATERIALS AND METHODS

Collection of specimens and preparation of style extract

Specimens of *Arcuatula capensis* were collected during November 1984 from the Bot River estuary, near Kleinmond, on the southwest coast of South Africa. The mussels were obtained from dense beds of *Ruppia maritima*, by SCUBA diving to depths of 3 to 5m. The specimens were transported to the laboratory in estuarine water on ice, where the crystalline styles of 40 animals were removed, rinsed and homogenised in 2ml chilled 20mM phosphate buffer (pH 7.0) in a ground-glass tissue homogeniser. The homogenate was subsequently centrifuged for 15 minutes at 15000xg, and the supernatant extracted for use in carbohydrase assays. For the lysozyme assays, the same procedure was followed, but all equipment in contact with the styles was autoclaved before re-use.

Determination of style composition

The dry weight and protein content of the styles were determined for relation to their enzyme activity. The average mass of 40 freeze-dried styles was 0.159mg. The composition of the styles was determined by CHN-analysis on a Heraeus model CHN-Mikro Universal combustion analyser. The styles were composed as follows: 23.9% C, 3.8% H and 6.6% N. Protein content was then determined by multiplying the value obtained for the organic nitrogen by the factor 6.25 (Lucas & Newell 1984), to yield 41.25% protein (an average of 0.066mg protein per style). A high proportion of this protein is of a structural nature (Seiderer *et al*. 1982) and direct comparisons with typical enzyme specific rates are therefore precluded. Before enzyme assays were performed, the protein content of the enzyme extracts was determined by the Lowry method (Lowry et al. 1951) and the concentrations were adjusted to 2mg protein.ml^{-1} , in phosphate buffer. This yielded protein concentrations of 0.082mg protein per style, which corresponds well with the results of the CHN analysis.

Carbohydrase activities.

While Breuil and Saddler (1985) consider the Nelson-Somogyi method to be superior to the DNS method for measurement of the release of reducing sugars during carbohydrase assays, Lucas and Newell (1984) and Fielding et al. (in prep.) have found the former to be the more sensitive. The activities of α-amylase, cellulase and laminarinase in the style extract were consequently determined using a modified Nelson-Somogyi method (Nelson 1944, Somogyi 1952) for measuring the liberation of reducing sugars by these enzymes from commercial substrates of 1% (w/v) starch, 1% (w/v) carboxymethylcellulose (CMC) and 0.4% (w/v) laminarin, respectively. Enzyme activities were also tested with a natural substrate of 1% (w/v) dried Ruppia maritima leaf detritus, obtained by ageing the leaves in fresh estuarine water for 6 months, in a shaking conical flask. The detritus was subsequently filtered off using pre-ashed GFC filters, dried at 60 °C and then homogenised and re-suspended in phosphate buffer before the assays were performed.

The release of reducing sugars was determined in a series of 6 incubations of increasing duration: 2 to 20 minutes, for the commercial substrates and 2 to 120 minutes, for the aged leaf material. In each case, a mixture of 0.5ml style extract (2mg protein.ml^{-1}) and 0.5ml substrate was incubated for the required duration in a shaking water bath at 20°C. Following the addition of 1ml of Somogyi reagents A and B (4:1), the mixture was boiled for 10 minutes. After cooling under running water, 2ml Nelson reagent was added, followed by 6ml distilled water. Colour was developed over 20 minutes and after centrifugation at 800xg for 5 minutes, the absorbance of the supernatant was read at 660nm, using a Beckman 25 Spectrophotometer.

Although the exact nature of the reducing sugars is not yet known, Fielding _et al_. (in prep.) note that better agreement between the DNS and Nelson-Somogyi methods is obtained when a maltose standard is used, instead of the customary glucose standard,which, they suggest, is likely to lead to an underestimation of the amount of reducing sugars released. Enzyme activities were therefore calculated from the calibration equation: $y = -0.02 + 6.24x$ ($r^2 = 0.99$; $n = 5$), where y is the absorbance at 660nm and x is the mass of maltose in milligrams (Fielding _et al_. (in prep.)). Absorption readings were corrected by subtraction of a phosphate buffer blank, and the mass of the reducing sugars released is given as the mean of triplicate determinations. Specific enzyme activities are given in mg maltose/mg style protein/hour.

Lytic enzyme activities

The presence of lytic enzymes in the crystalline style of _Arcuatula capensis_ was investigated by assaying the action of the enzyme extract on bacteria isolated from the style, as well as from estuarine water (free-floating bacteria).

a) Isolation of bacteria

The crystalline styles of 5 specimens were extracted in the most sterile manner possible, and homogenised in a ground-glass tissue homogeniser in 0.5ml phosphate buffer. Subsamples of 0.1ml from a tenfold serial dilution of the homogenate were pipetted onto agar plates made of 2% agar in a growth medium of 0.5% (w/v) peptone (DIFCO) and 0.1% yeast extract, in a mixture of 50% distilled water and 50% 0.45μm filtered, sterilised seawater. Fifty percent dilution of seawater was used, since Roberts et al. (1985b) report maximum growth rates of Bot River estuarine bacteria at a salinity of 17‰. The plates were incubated for 48 hours at 25 °C. Bacterial strains were subsequently restreaked to obtain isolated colonies, and the pure cultures stored on 50% seawater agar plates.

Free-floating bacteria were isolated from water samples taken near the bottom in the Bot River estuary, with sterile glass vials. Subsamples (0.1ml) of a tenfold serial dilution of the estuarine samples were pipetted onto 50% seawater agar plates, and pure bacterial cultures isolated in the manner described above.

b) Assay of the lytic enzyme(s)

Agarose plates containing heat-killed bacteria from the crystalline style of the bivalve or from the estuarine water, were used independently to determine the presence and activity of lytic enzymes from the style, following the procedure below.

Bacterial isolates were lifted off the agar plates and incubated for 10 hours on an orbital shaker, in a nutrient broth containing 0.5% (w/v) peptone (DIFCO) and 0.1% (w/v) yeast extract in 50% seawater (McHenery et al. 1979, Seiderer et al. 1984). After centrifuging the bacterial suspensions at 5000xg for 10 minutes, the bacteria were re-suspended in 5ml sterile estuarine water and the optical density of a 1:10 dilution determined at 600nm.

The bacterial suspensions were heated for 15 minutes in a water bath at 65 °C, to kill the bacteria. Using the optical densities to standardise concentrations, aliquots of the heat-killed bacteria were pipetted into 100ml agarose medium (0.8% (w/v) agarose in sterile estuarine water) at 65 °C. This mixture was then divided equally between five 84mm diameter sterile petri dishes (20ml each). After cooling, seven wells of equal diameter were punched into the agarose plates, for reception of the style enzyme extract. Tenfold serial dilutions of the enzyme extract were made up, and a 15µl subsample of each dilution was pipetted into six of the wells. The seventh well received 15µl sterile phosphate buffer as control. The plates were then incubated for 48 hours at 25 °C. Zones of lysis were subsequently made visible by staining the intact bacteria with 2ml crystal violet (0.12%). After rinsing with distilled water and measuring the diameters of the zones, the number of bacteria lysed in each zone over the 48 hours could be calculated from the initial volume of bacterial suspension introduced into the agarose medium, its optical density (1 ODU = 8x10 cells.ml^{-1}), and the area of lysis. Specific enzyme activity is given as the number of bacteria lysed per milligram of style protein per hour.

RESULTS AND DISCUSSION

Carbohydrase activities

The enzyme activities of the carbohydrases α-amylase, laminarinase and cellulase in the crystalline style of Arcuatula capensis are given in Figures 1 to 3, and their specific activities in Table 1.

Figure 1 shows that the style enzymes have high α-amylase activity, indicated by the release of maltose from a commercial starch substrate. The reaction remained unsaturated after 20 minutes. Gianfreda et al. (1979) have found that α-amylase is the major glucanase in 14 marine molluscs, but that its activity is not related to the feeding habits of these species. The high activities of the enzyme noted by these authors, and also described here for Arcuatula capensis, are probably explained by the widespread occurrence of starch and glycogen in the food of bivalves.

Lower, but significant laminarinase activity was detected in the style extract, using laminarin as a substrate (Fig. 2). In an estuarine environment the detrital food is highly refractory and even low laminarinase activities may be important for the digestion of chrysolaminarin in diatoms, thereby making it available as an energy source. In a survey of laminarinases in 50 marine invertebrates, Sova et al. (1970) have recorded the highest activities in crustaceans and molluscs. Although the activities depended more on the systematic position of the species than on their diet, it was found that feeding habits played an important role within taxonomic classes. Kristensen (1972) has similarly

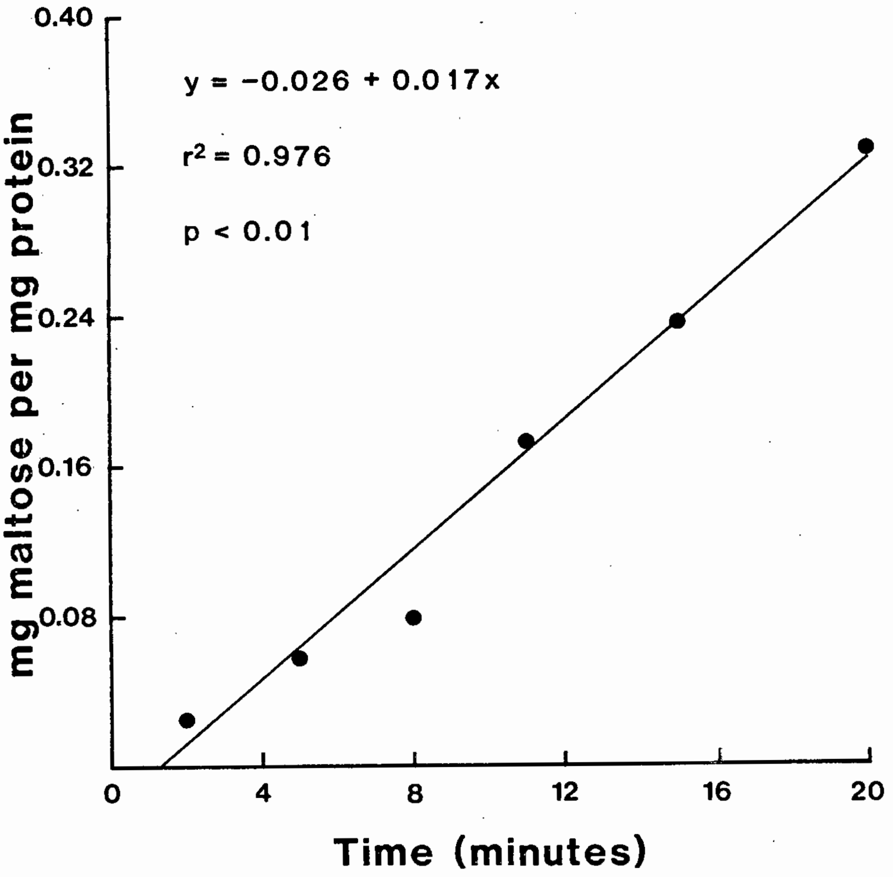

Figure 1: The activity of 2mg.ml^{-1} enzyme extract from the crystalline style of Arcuatula capensis on a 1% (w/v) starch substrate at 20 °C. The release of maltose was measured in 6 triplicate incubations of between 2 and 20 minutes.

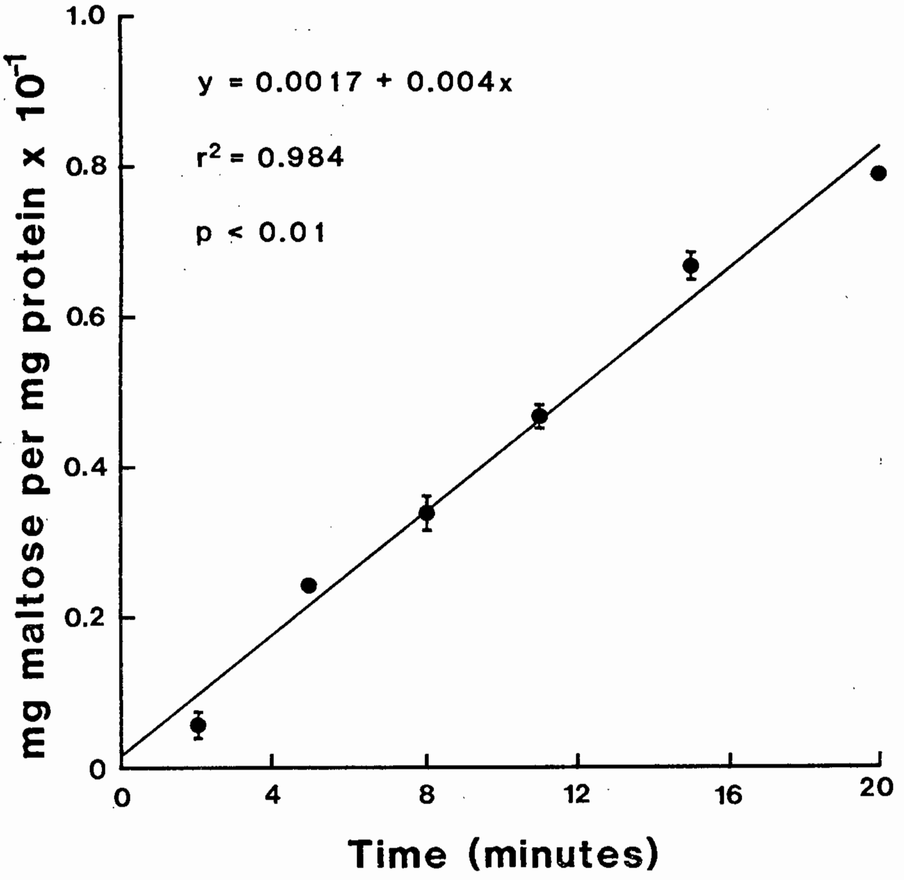

Figure 2: The activity of 2mg.ml^{-1} enzyme extract from the crystalline style of *Arcuatula capensis* on a 0.4% (w/v) laminarin substrate at 20 °C. The release of maltose was measured in 6 triplicate incubations of between 2 and 20 minutes.

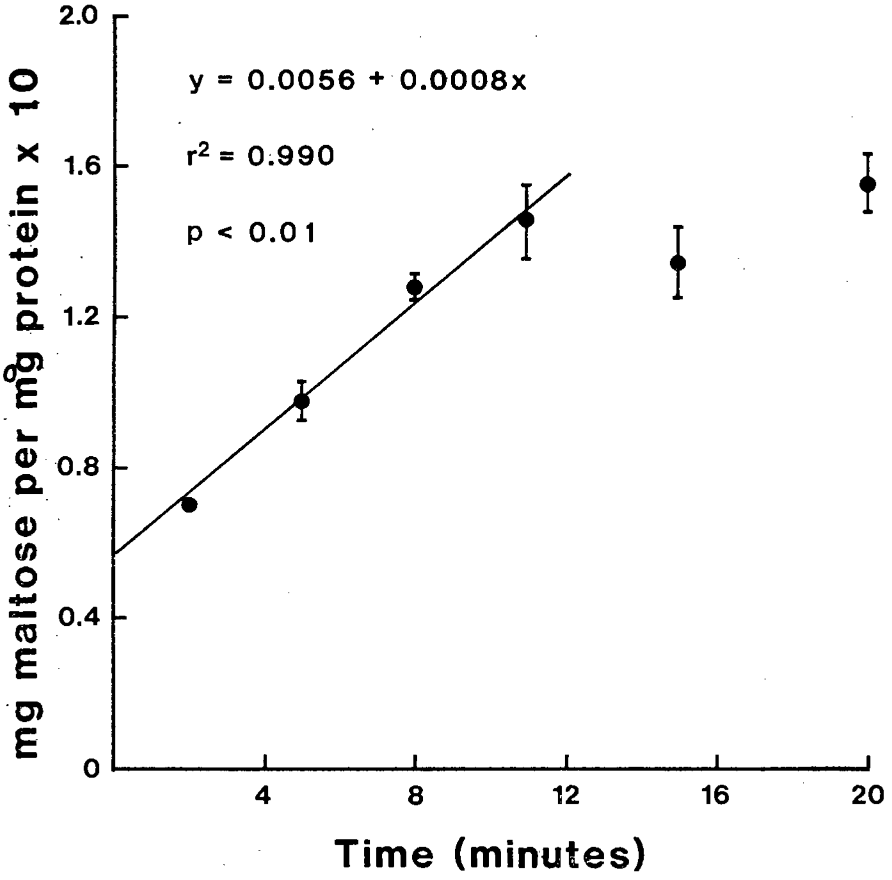

Figure 3: The activity of 2mg.ml^{-1} enzyme extract from the crystalline style of *Arcuatula capensis* on a 1% (w/v) CMC substrate at 20 °C. The release of maltose was measured in 6 triplicate incubations of between 2 and 20 minutes.

Table 1: Specific activities of three carbohydrases of the crystalline style of Arcuatula capensis, in terms of the amount of maltose released per mg style per hour and per mg style protein per hour from the assay substrates, starch, CMC and laminarin.

Enzyme	mg maltose/ mg style/h	mg maltose/ mg protein/h
α-amylase	0.432	1.046
cellulase	0.021	0.051
laminarinase	0.101	0.244

noted a correlation between diet and the presence of laminarinases in 22 marine invertebrates.

Lowest activities in the style extract were measured (Fig. 3) for cellulase, using carboxymethylcellulose (CMC) as a substrate. Activity was only linear in time for the first 11 minutes of incubation, thereafter slowing down, presumably due to low levels of the enzyme. The presence of style cellulases was first reported in clams by Lavine (1946). Since then, it has been shown that many molluscs contain endogenous (non-bacterial) cellulases (Elyakova 1972, Foulds & Mann 1978). Wood (1975) has shown, however, that the ability to digest CMC does not necessarily indicate an equal ability with natural substrates, and this was found to be the case for the style enzymes of *Arcuatula capensis*, which failed to release any reducing sugars from aged leaves of *Ruppia maritima*. Crosby and Reid (1971) have reported a general correlation between cellulase activities in nine bivalve species, and the cellulose content of their diet, but found that the highest level of cellulose digestion did not occur in contact with the style, but in the digestive diverticula.

Mann (1982) and others (Kristensen 1972, Tenore 1981) have pointed out that invertebrates do not generally digest the structural polysaccharides found in macrophytic detritus. Instead, the leaf material is first degraded by micro-organisms, which are themselves subsequently consumed by the invertebrates. Stuart *et al.* (1982), however, have shown that the marine bivalve, *Aulacomya ater*, can obtain carbon directly from kelp debris. This is less likely to occur with the more refractory detritus found in estuaries (Tenore *et al.* 1979). The estuarine bivalve, *Arcuatula capensis*, probably depends on bacteria for the initial degradation

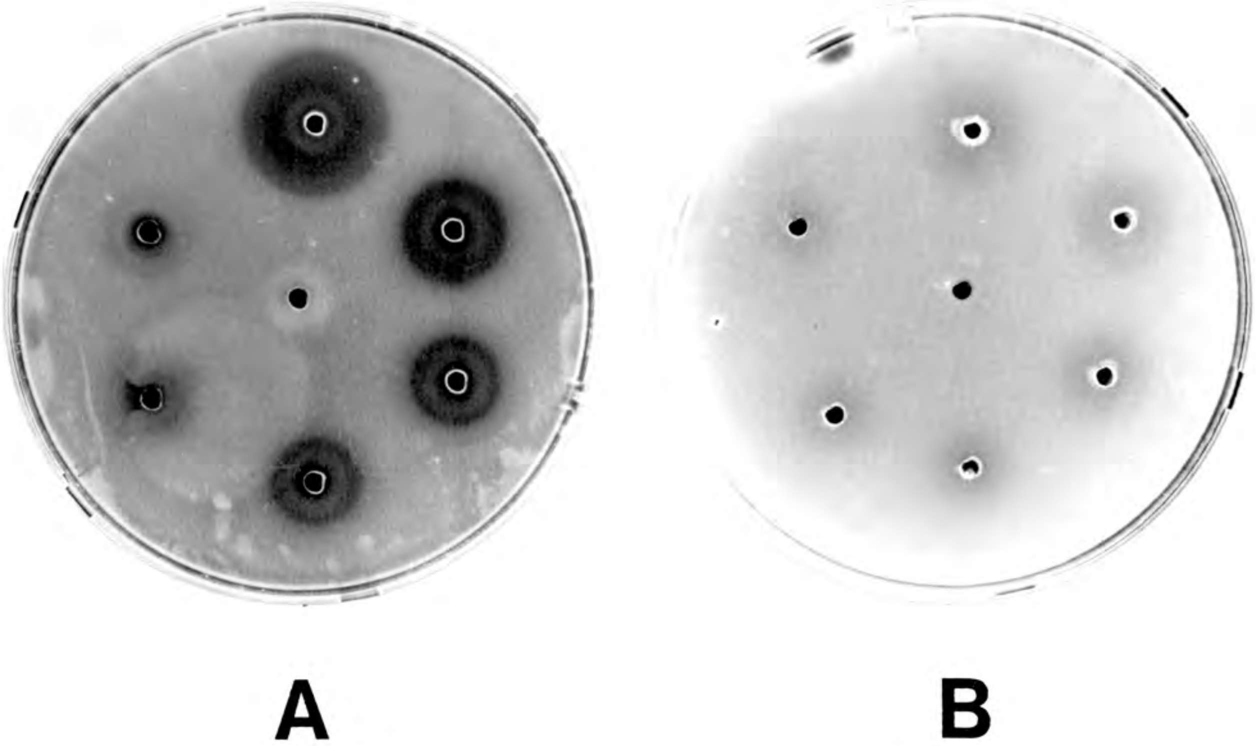

Figure 4: The lytic zones produced by the action of 6 tenfold serial dilutions of a 2mg.ml^{-1} enzyme extract from the crystalline style of Arcuatula capensis, on a free-living, heat-killed bacterial strain suspended in an agarose medium (A). A second strain shows no susceptibility to lysis (B). The central hole received phosphate buffer only, and serves as a control.

of leaf material and the accompanying release of intracellular polysaccharides, which are digestible by its α-amylase enzymes. The microbes themselves may also constitute a food source if the bivalve possesses the lytic enzymes necessary to digest them.

Lytic enzymes

The presence of lytic enzymes in the crystalline style of *Arcuatula capensis* was indicated by the action of the style extract on bacterial strains isolated from estuarine water and from the style itself. Lytic activities are evident from the zones on the agarose plate shown in Figure 4A, which contains a suspension of free-floating bacteria isolated from the estuarine water. This plate is compared with an agarose plate containing a different free-floating bacterial strain (Fig. 4B), in which no lysis has occurred. Prieur (1981) has shown, using electron microscopy of the digestive tract of the bivalve *Mytilus edulis*, that certain bacterial strains are digested more rapidly than others, while some strains (especially those of the genus *Vibrio*) are completely resistant to digestion.

Figure 5 gives the power regressions for the number of bacteria lysed per millilitre of enzyme extract versus the protein concentration of the extract. Four bacterial strains are compared - two isolated from estuarine water and two from the style. The regression statistics are recorded in Table 2. The slopes of the regressions represent the number of bacteria lysed per milligram protein, and therefore give an indication of the susceptibility to lysis of each bacterial strain. The statistical significance of the differences between the slopes of these four strains is given

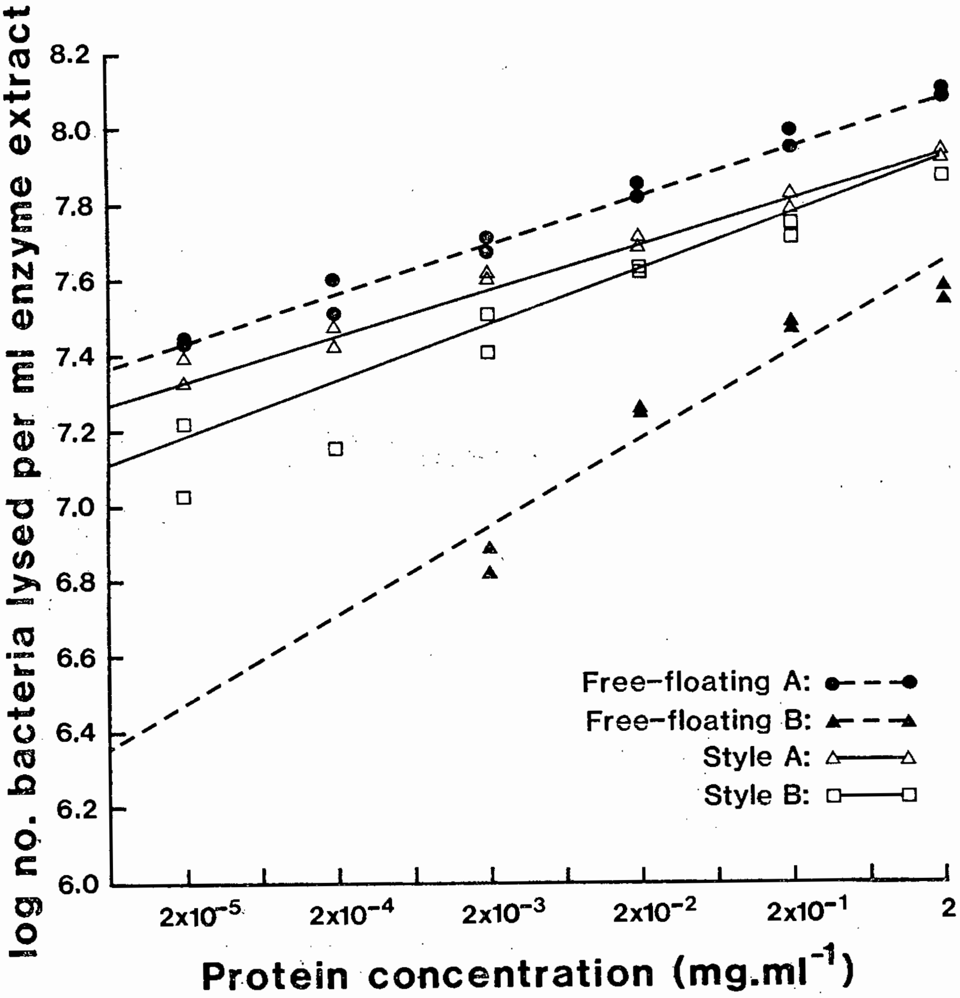

Figure 5: Power regressions for the lytic activities of 6 tenfold serial dilutions of a 2mg.ml^{-1} enzyme extract from the crystalline style of Arcuatula capensis, on 4 bacterial strains, 2 isolated from the style (Style A & B) and 2 from estuarine water (Free-floating A & B). The regression statistics are given in Table 2.

Table 2: Statistics of the power regressions ($y = ax^b$) given in Figure 5, for the lytic activity of _Arcuatula capensis_ crystalline style extract on 2 style and 2 free-floating bacterial strains (y=number of bacteria lysed per ml enzyme extract; x=mg protein per ml enzyme extract).

Bacterial isolates	a	b	r^2	n
style A	7.62×10^7	0.116	0.938	12
style B	7.46×10^7	0.185	0.929	10
free-floating A	1.12×10^8	0.131	0.994	12
free-floating B	3.66×10^7	0.234	0.918	8

Table 3: The t-statistics for comparisons between the slopes of the regressions in Figure 5 and Table 2. Significant differences ($p < 0.01$) are indicated by asterisks (degrees of freedom are given in parentheses).

Bacterial isolates	style A	style B	free-floating A
style B	3.610(18)*	-	
free-floating A	1.524(20)	3.450(18)*	-
free-floating B	3.988(16)*	1.500(14)	5.025(16)*

in Table 3. No clear separation can be detected between the free-floating and style bacteria. Corresponding variation is apparent in the specific activities of the enzyme extract assayed with these 4 isolates (Table 4).

Assuming that bacteria represent an important nutritional resource for Arcuatula capensis, factors which adversely affect bacterial densities can be expected to have a deleterious effect on the bivalve population. Roberts et al. (1985b) have shown that the optimal salinity for free-floating bacteria in the Bot River estuary is 17‰. Very low salinities (<3.5‰), which occur during periods of high water levels, severely inhibit growth of these bacteria. Since bacteria also play an important role in the degradation of Ruppia maritima (Roberts et al. 1985a), the bivalves may become both nitrogen- and energy-limited during extended periods of low salinities. Tenore (1983), for example, has found that growth of the polychaete, Capitella capitata, could be energy-limited by the rate of bacterial degradation of refractory detritus. The presence of a laminarinase in the crystalline style of the bivalve, however, allows it to digest the chrysolaminarin of diatoms. Although phytoplankton levels are fairly low in the estuary, this additional food resource may become important during times of low bacterial biomass.

These biochemical mechanisms provide Arcuatula capensis with the means by which the variable food resources of the Bot River estuary can be utilised, and may represent a necessary pre-adaptation for colonisation of this stressful environment.

Table 4: Specific activities of the lytic enzymes of the crystalline style in Arcuatula capensis, assayed with 4 bacterial strains: 2 from the style and 2 from estuarine water. The values given represent the mean of 2 determinations.

Bacterial strain	Bacteria lysed/ mg style/h	Bacteria lysed/ mg protein/h
style A	8.29×10^{9}	2.16×10^{10}
style B	5.49×10^{9}	1.33×10^{10}
free-floating A	1.12×10^{10}	2.70×10^{10}
free-floating B	5.13×10^{5}	1.24×10^{6}

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CHAPTER FIVE

The presence of a lytic enzyme in the estuarine isopod, _Exosphaeroma_ _hylecoetes_ (Barnard)

ABSTRACT

Although the importance of bacteria in the diet of marine and estuarine invertebrates has been recognised for some time, the presence of lytic enzymes, which digest the bacteria, has been shown in bivalves only. In this paper, ingestion of bacteria by the estuarine isopod, Exosphaeroma hylecoetes (Barnard), is indicated by electron microscopy of the foregut. The presence of lysozyme is then demonstrated by the lysis of bacterial suspensions in agarose, by enzyme extracts from the isopod. Not all of the bacterial strains tested were lysed by the extract, while those that were, showed varying degrees of sensitivity to the enzyme(s).

INTRODUCTION

Since it was first shown that certain bivalves can filter bacteria from the water column (Zobell & Landon 1937, Zobell & Feltham 1938), it has become well known that micro-organisms form an important food resource to many detritivores. Numerous studies have established the role of bacteria as a nutritional source *per se* (Lopez *et al.* 1977, Wright *et al.* 1982), as gut flora able to digest cellulose (Wainwright & Mann 1982), or as colonisers and degraders of macrophytic debris, making the detrital carbon available to macroconsumers (Newell 1965, Tenore 1983). While it has also become clear that certain marine invertebrates can obtain sufficient carbon directly from algal debris (Hanson 1982, Stuart *et al.* 1982), detritivores in estuaries tend to be dependent on micro-organisms for release of carbon from the more refractory debris found there (Findlay & Tenore 1982).

Only recently, however, was the existence of lytic enzymes in marine bivalves shown to be important for the digestion of bacteria as food (McHenery *et al.* 1979), as opposed to their postulated use as a defence mechanism (McDade & Tripp 1967, Hardy *et al.* 1976). Seiderer *et al.* (1984) have found that the crystalline style of *Choromytilus meridionalis* contains an enzyme which lyses most free-living bacteria occuring in the near-shore environment. Chapter Four similarly demonstrates the presence of a lysozyme in the style of an estuarine bivalve, which is capable of lysing free-living estuarine bacteria. No record exists of a lysozyme in any crustacean, however, although the importance of bacteria in their diet is well established (Baker & Bradnam 1976, Lopez *et al.* 1977, Moriarty 1977). This study demonstrates, for the first time, the presence of a lytic enzyme in a benthic estuarine crustacean - the isopod, *Exosphaeroma hylecoetes* (Barnard).

MATERIALS AND METHODS

Collection of specimens and preparation of enzyme extract

Specimens of *Exosphaeroma hylecoetes* were collected during November 1984, from beds of *Ruppia maritima* in the Bot River estuary, near Kleinmond, on the southwest coast of South Africa. The isopods were transported in estuarine water on ice, to the laboratory, and freeze-dried. Lucas and Newell (1984) have shown that freeze-drying does not alter the activity of digestive enzymes. For enzyme extraction, freeze-dried specimens were homogenised under sterile conditions, in 2ml of chilled 20mM phosphate buffer (pH 7.0) in a ground-glass tissue homogeniser, and subsequently centrifuged at 15000xg for 15 minutes. The supernatant was extracted, its protein content determined by the method of Lowry *et al.* (1951), and the concentration adjusted to 1mg protein ml^{-1} phosphate buffer. This was stored at 4 °C until used (always within 5 days). All equipment and solutions in contact with the extract were autoclaved before use.

Photography of the gut by scanning electron microscopy

Isopod guts were prepared for scanning electron microscopy, to determine the presence of bacteria. Captive isopods, feeding on live *Ruppia maritima*, were dissected to remove their guts, which were then dehydrated through an ethanol series. Thereafter, the guts were critical-point dried, using a Polaron E3000 apparatus (Anderson 1951), and mounted on specimen stubs. An incision was made down the length of each gut in order to open and expose its interior. The stubs were then coated with a gold/palladium alloy in a Hummer vacuum-coating unit. The specimens were viewed, and selected areas photographed using a Cambridge S200 scanning electron microscope.

Lytic enzyme activity

The lytic enzyme activity of the extract was assayed by the lysis of target bacterial strains isolated from fresh Ruppia maritima leaf material and from estuarine water.

For this purpose, free-floating bacteria were isolated by the methods described in Chapter Four, from water samples taken with sterile glass vials near the bottom of the Bot River estuary. Fresh Ruppia maritima leaves were collected in the estuary, immediately placed into autoclaved estuarine water and transported to the laboratory. Here they were transferred to a nutrient broth containing 0.5% (w/v) peptone and 0.1% (w/v) yeast extract, in 0.45µm filtered, sterile seawater, mixed with an equal volume of sterile distilled water. Roberts et al. (1985) have shown that maximum growth rates of Bot River estuarine bacteria are obtained with culture media at a salinity of 17‰ (50% seawater). The nutrient broth containing the leaves was shaken at 25 °C until turbid (overnight). Subsamples (0.1ml) of a tenfold serial dilution of the bacterial suspension were pipetted onto 50% seawater agar plates, and the bacterial strains isolated and stored, as described in Chapter Four.

Agarose plates containing heat-killed bacteria from the estuarine water and from the Ruppia maritima leaves were used to determine the presence and activity of lytic enzymes from Exosphaeroma *hylecoetes*. The methods used follow those of Seiderer *et al*. (1984) and are given in Chapter Four.

RESULTS AND DISCUSSION

Figure 1 is a micrograph of the foregut of the isopod, *Exosphaeroma hylecoetes*. Bacteria are clearly visible on the wall of the gut. Newell and Field (1983) have shown that bacteria may constitute an important nitrogen resource for animals which are able to digest them. Prieur (1981) has used micrographs to indicate the extracellular digestion of bacteria in bivalves, while Boyle and Mitchell (1978) have suggested that the absence of bacteria in the digestive tracts of two marine wood-boring isopods probably indicates the lysis of ingested bacteria. In Figure 2, which is a micrograph of the hindgut, no bacteria can be seen, and it seems reasonable to assume that they are digested by the isopod. A more conclusive method, however, is to determine whether or not the animal possesses the enzyme(s) necessary for the digestion of bacteria.

In Figure 3, the lytic zones produced by the action of a tenfold serial dilution of enzyme extract from the isopod, on a suspension of a bacterial strain in agarose can be seen. These zones correspond well with those obtained by Seiderer *et al*. (1984) (and with those described in Chapter Four), for lytic enzymes in bivalves, and consequently indicate that *Exosphaeroma hylecoetes* has the ability to digest bacteria. The enzyme extract did not produce lytic zones with all the bacterial isolates, however, supporting the results of Prieur (1981), who also noted varying susceptibilities to lysozyme in different strains.

Variation in sensitivity to lytic enzymes is evident from the regressions (Fig. 4), which have been calculated from the lysis of four bacterial suspensions, two of which were isolated from *Ruppia*

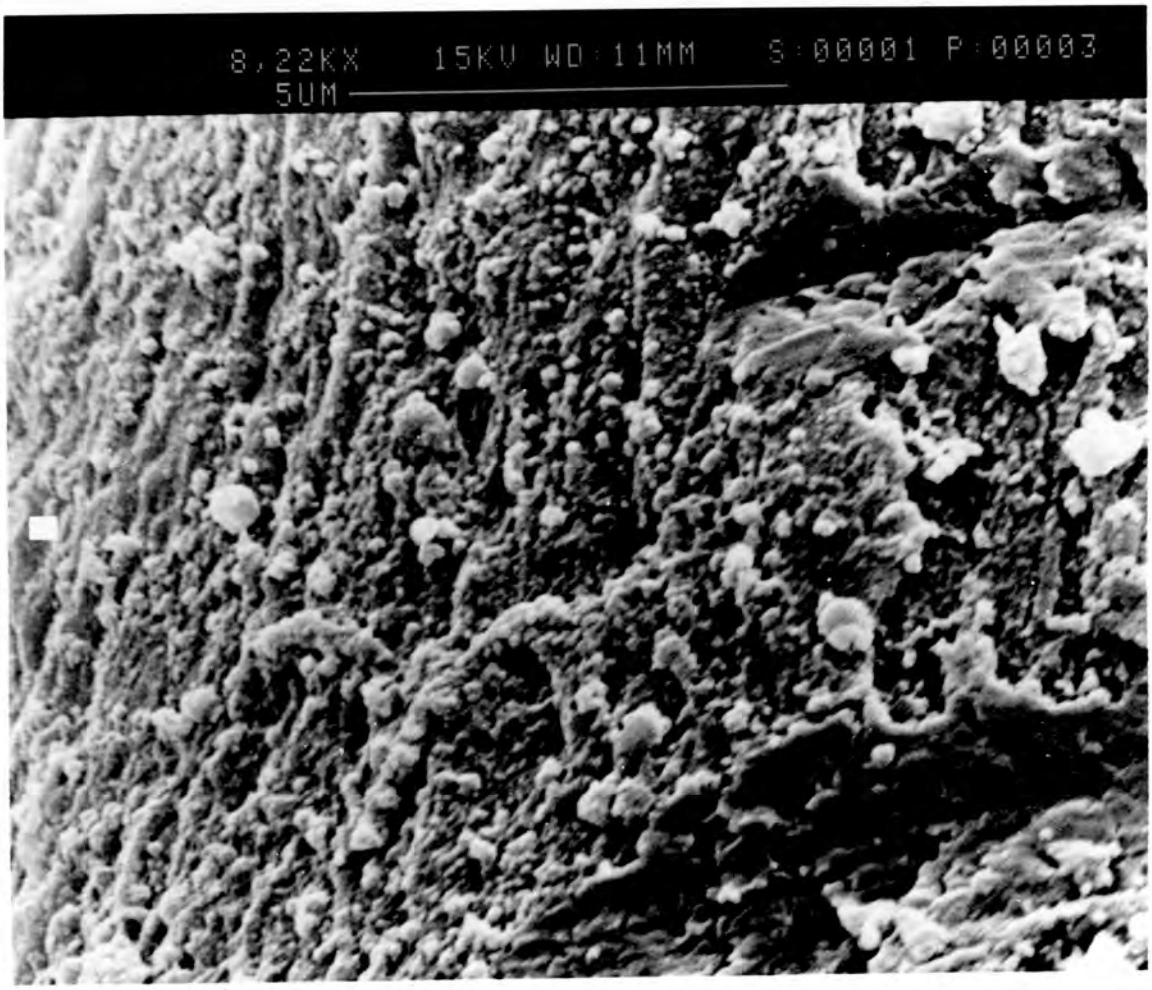

Figure 1: Scanning electron micrograph of the foregut of Exosphaeroma hylecoetes, showing the presence of bacteria.

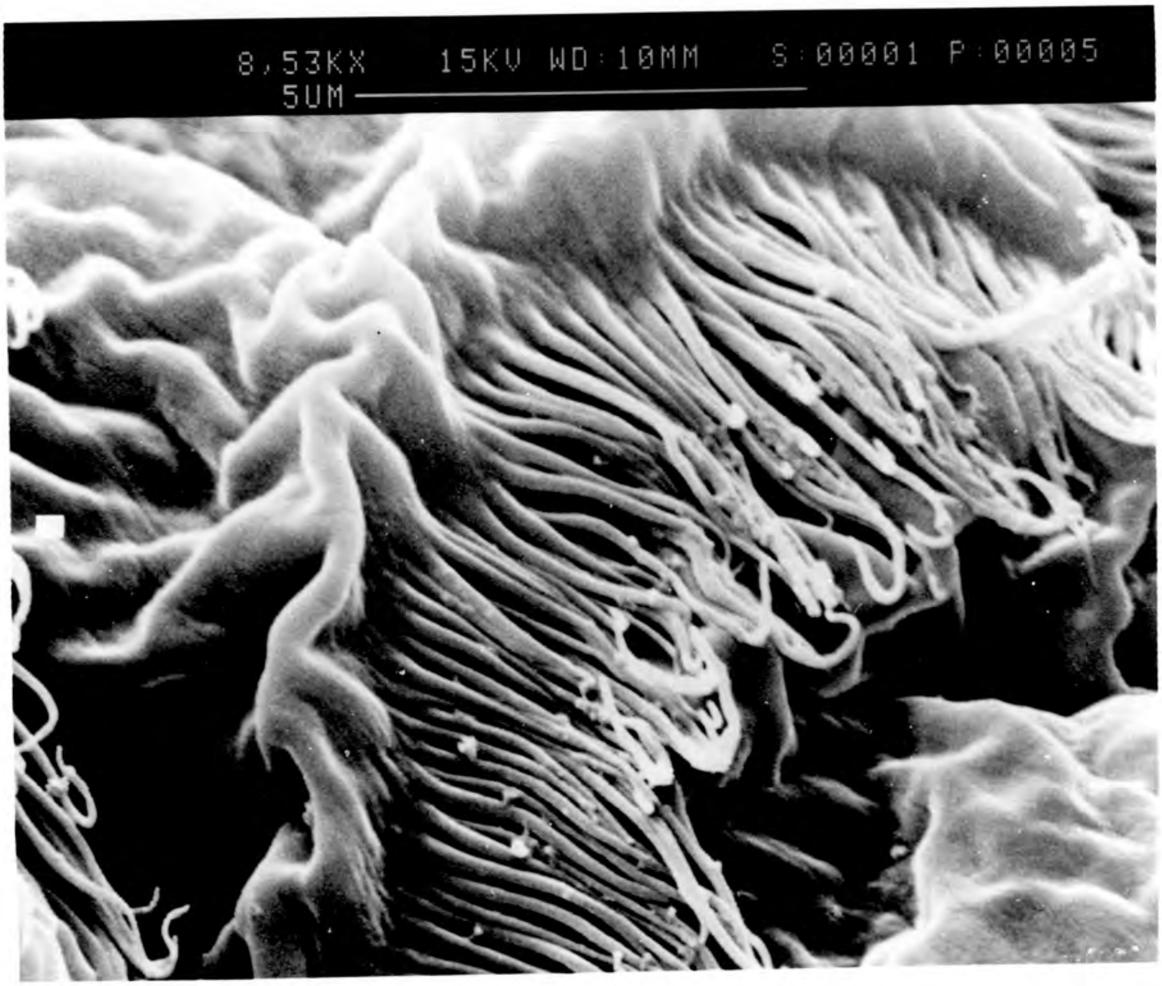

Figure 2: Scanning electron micrograph of the hindgut of Exosphaeroma hylecoetes. No bacteria can be seen.

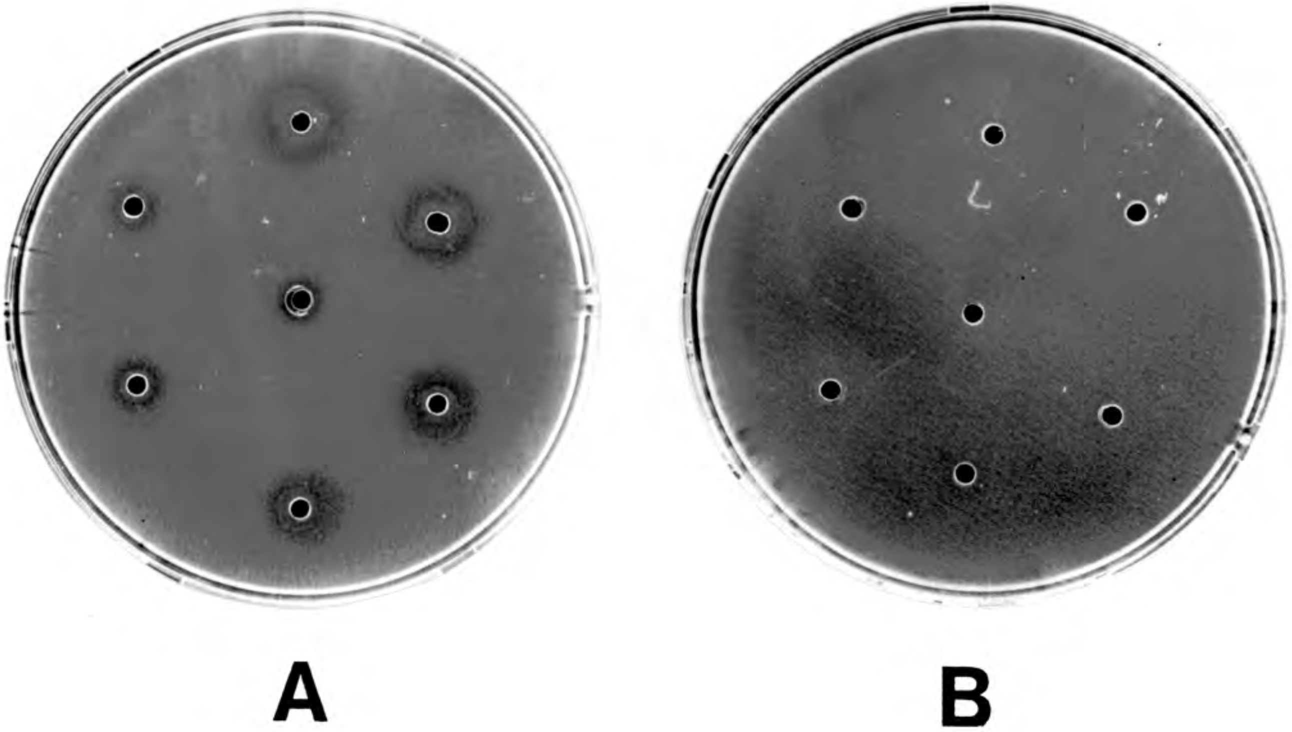

Figure 3: The lytic zones produced by the action of 6 tenfold serial dilutions of a $1 mg.ml^{-1}$ enzyme extract from the isopod, *Exosphaeroma hylecoetes*, on a free-living, heat-killed bacterial strain suspended in an agarose medium (A). A second strain shows no sensitivity to lysis (B). The central hole received phosphate buffer only, and serves as control.

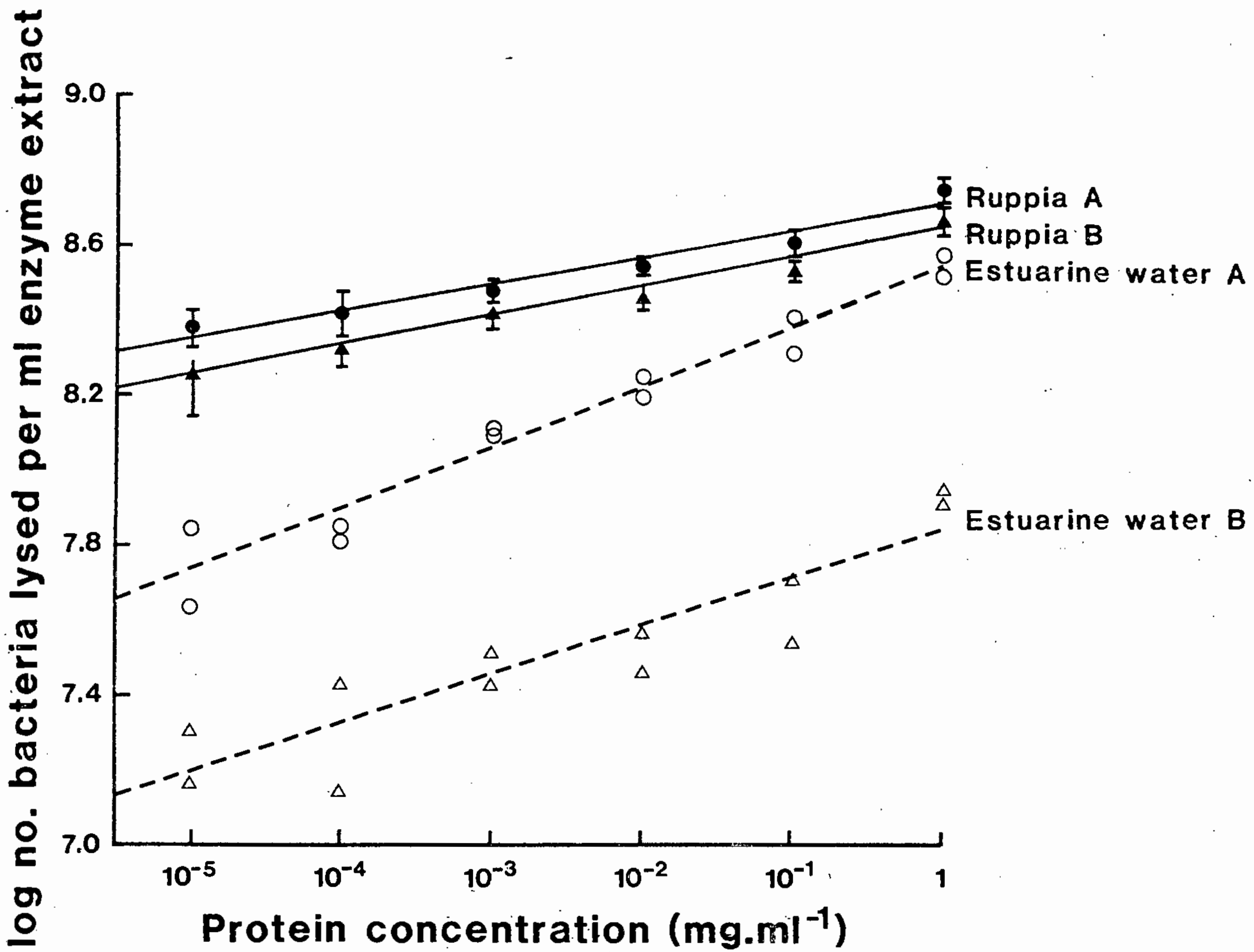

Figure 4: Power regressions of the number of bacteria lysed versus the protein concentration of an enzyme extract obtained from <u>Exosphaeroma hylecoetes</u>. Lytic activity was tested on 4 bacterial strains: 2 isolated from <u>Ruppia maritima</u> leaves (<u>Ruppia</u> A & B) and 2 from the water of the Bot River estuary (Estuarine water A & B). The regression statistics are given in Table 1.

leaves, and two from estuarine water. The regression statistics are given in Table 1. Since the slopes represent the number of bacteria lysed per milligram protein, their different values give an indication of the variation in susceptibility of the bacterial strains to the lytic enzymes. As only two bacterial strains were chosen from each bacterial population to test for lytic activity, it is impossible to judge whether one population is more sensitive to lysis than the other.

In conclusion, it is clear that _Exosphaeroma hylecoetes_ not only ingests bacteria, but that it also has the ability to digest them. Although the bacteria show differing sensitivities to the lytic enzymes, they may represent an important nitrogen resource for the isopod. This is especially important in an estuarine environment in which the C:N ratios of the available resources are typically very high (Newell & Field 1983). The presence of a lytic enzyme may therefore be crucial to the survival of this species in estuaries.

Table 1: Statistics of the power regressions ($y = ax^b$) given in Figure 4, of the number of bacteria lysed versus the protein concentration of an enzyme extract obtained from *Exosphaeroma hylecoetes*. Lytic activity was tested on 4 bacterial strains: 2 isolated from *Ruppia maritima* leaves (*Ruppia* A & B), and 2 from water of the Bot River estuary (Estuarine water A & B).

Bacterial strain	a	b	r^2	n
Ruppia A	4.76×10^8	0.064	0.965	6
Ruppia B	4.42×10^8	0.080	0.976	6
Water A	3.55×10^8	0.165	0.989	6
Water B	6.84×10^7	0.126	0.927	6

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CHAPTER SIX

A comparison of the physiological condition of the southern mullet, *Liza richardsoni* (Smith), in a closed estuary and the sea.

by

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A COMPARISON OF THE PHYSIOLOGICAL CONDITION OF THE SOUTHERN MULLET *LIZA RICHARDSONI* (SMITH), IN A CLOSED ESTUARY AND THE SEA

By

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SUMMARY

The physiological condition of the mullet, *Liza richardsoni* (Smith), from the closed Bot River estuary is compared with that of the same species from False Bay. Levels of protein, lipid, water and ash, as well as energy content were determined in fish from the two environments. Protein (17,94 % *vs.* 18,53 %) and ash levels (3,41 % *vs.* 4,81 %) were similar in both estuarine and marine fish. Lipid (9,95 % *vs.* 2,23 %) and energy levels (18,35 kJ g^{-1} dry weight *vs.* 14,18 kJ g^{-1} dry weight), were significantly higher, while levels of water (67,73 % *vs.* 74,87 %) were lower in the estuarine fish. On average the estuarine *L. richardsoni* were in better condition than their marine conspecifics.

Liza richardsoni does not spawn in estuaries and their better condition in the estuary could possibly be attributed to the retention of energy usually lost through spawning in the sea. On the other hand, estuarine subadults were already in an improved condition, which could only be due to better feeding conditions. It is therefore concluded that estuarine *L. richardsoni* can maintain a better condition due to both more favourable feeding conditions and the retention of energy usually lost through spawning.

INTRODUCTION

Estuaries have long been known to be organically rich in comparison with marine coastal systems (Odum & De la Cruz 1967, Head 1976, Day 1981). Many South African estuarine fish species are marine migrants, presumably utilising the abundant food sources available in estuaries (Blaber 1981, Day *et al.* 1981). Since the condition of fish has been directly related to their food selection (Blaber 1975), it follows that if estuarine fish benefit from the richer feeding grounds, they should be in better physiological condition than their marine conspecifics. This, however, has not yet been shown by a direct comparison of estuarine and marine fish populations.

The commercial importance of mullet (Mugilidae) has given rise to a large literature on their physiological condition. Attention has generally focussed on the grey mullet, *Mugil cephalus* (Linnaeus), since it is cultured in many parts of the world. Chemical analyses of the tissues of this species are readily available (for reviews see: Thomson 1966, Love 1970, 1980), but no comparisons between marine and estuarine mullet could be found.

Relatively few studies have dealt with the southern mullet, *Liza richardsoni* (Smith), since it has a limited range, extending from Moçamedes, in southern Angola, to Transkei, on the east coast of the Republic of South Africa (Penrith 1978). Marais (1976) and Marais & Erasmus (1977) reported analyses of *L. richardsoni* caught in the Swartkops Estuary near Port Elizabeth, but did not include comparisons with marine mullet. As far as could be ascertained, their study and that of Van Wyk (1944) are the only analyses of *L. richardsoni* published to date.

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In this paper, the physiological condition of estuarine and marine *L. richardsoni* is compared in order to determine whether richer feeding areas contribute to better condition in estuarine mullet.

Estuarine *L. richardsoni* were obtained from the Bot River estuary, near Kleinmond on the south-west coast of South Africa (34°20′S 19°06′E). A detailed description of the estuary may be found in Koop *et al.* (1983). Pertinent to this study is the fact that the estuary is normally separated from the sea by a sand barrier, but that at high water-level, a natural overflow channel opens to the sea via the swamps at Kleinmond. Due to the water-releasing effect of this channel, the sand barrier is seldom breached naturally, and at the time of sampling (April 1981), the estuary had been closed for four years (1977–1981). Since the overflow channel allows only limited recruitment of fry at times of high water-level, the estuarine *L. richardsoni* were effectively trapped inside the estuary during this four-year period. This afforded the ideal opportunity for comparison with marine mullet, which was caught in False Bay, approximately 50 km west of the Bot River estuary.

MATERIALS AND METHODS

Collection of samples

Samples of estuarine *L. richardsoni* were obtained in April 1981 in the Bot River estuary, using a small seine net (25 m × 2 m; 10 mm stretched mesh) and braided nylon gill nets (760 m × 2,2 m; 8 mesh sizes ranging from 35 mm to 197 mm stretched mesh). In False Bay, marine fish were caught by dragging the small seine net at St James and Fish Hoek beaches (34°07′S 18°28′E) to catch juveniles and fry, while larger fish were obtained from commercial treknet fishermen at Strandfontein (34°05′S 18°40′E). All fish were measured (total length: TL) and assigned to one of seven 60 mm size-classes (0–420 mm). They were frozen immediately thereafter and stored at −20 °C until processed.

The abundance of fish of various size-classes differed in the Bot River estuary and False Bay. In the estuary, the fish of the smaller size-classes (TL <180 mm) were scarce, while in False Bay no fish of the two largest size-classes (TL >331 mm) were caught.

Analyses of body composition

Since the proportions of body constituents vary in different parts of the body (Suppes *et al.* 1967), whole fish were used in composition analyses. After the contents of their alimentary canals had been removed, fish were homogenized together in each size-class. The larger size-class (TL >180 mm) were represented by three randomly selected fish, while all the fish in the smaller size-classes were homogenized. Fish were minced in a Spong no. 5 meat mincer and after thorough mixing, homogenized with an Ultra-Turrax homogenizer. Homogenates were stored at −20 °C until processed further.

Subsamples of the homogenates of each size-class were analysed for protein, water, lipid and ash levels (expressed as % of total wet weight), as well as for energy content (expressed as kJ per gram dry weight). The levels were determined in the following ways:

(i) protein: subsamples were analysed for total protein (total nitrogen × 6,25) by the micro-Kjeldahl method (Kirk 1947).

(ii) water: levels were determined as weight loss from preweighed subsamples, oven-dried at 65 °C to constant weight.

(iii) lipid: total lipids were determined in subsamples by a method adapted from Bligh & Dyer (1959), using methanol and chloroform as extraction solvents.

(iv) ash: subsamples were ashed for 5 hours in a muffle furnace at 450 °C.

(v) energy: subsamples of approximately 0,5 g dry weight were analysed in a Gallenkamp bomb calorimeter.

RESULTS

Body composition

Table 1 gives the levels of the four body constituents and the energy content in each size-class. The levels of protein were generally similar in both estuarine and marine mullet, while ash levels were higher in the marine fish, possibly due to their sandier feeding substrate. Differences in the levels of lipid, water and energy, however, were more pronounced between the two stocks. Lipid levels were higher in all the estuarine mullet, with the biggest variations (up to 15 %) occurring in the larger size-classes. Energy-content followed the same trends, with differences of up to 7 kJ g^{-1} dry weight. The opposite was true for the water-levels, which were higher in all but one of the size-classes of marine mullet. The biggest variations in the water-levels (up to 14 %) were again found in the larger mullet.

Significant negative correlations exist between lipid and water-levels ($r = -0{,}99$; $p<0{,}001$), as well as between energy-content and water-levels ($r = -0{,}97$; $p<0{,}001$) in the estuarine mullet. A significant positive correlation was found to exist between the lipid and energy-levels ($r = +0{,}98$; $p<0{,}001$) in the estuarine fish. None of these constituents were significantly correlated in the marine mullet.

Changes in composition with length

It is clear from Table 1 that there are far greater variations in lipid, water and energy between size-classes in the estuary than in the sea. Figure 1 illustrates the changes in body constituents with length in the mullet from the two environments. It is apparent that the increase in lipid in the larger size-classes of estuarine fish is associated with a relative decrease in protein and ash-levels (Fig. 1A).

The changes in lipid, energy and water with length can be more readily compared in Figures 2, 3 and 4. Levels of significance were determined using Student's t-test. It can be seen that while lipid, energy and water are significantly correlated with length in the estuarine mullet, only energy is significantly correlated with length in the marine mullet. The regressions of length *vs*. lipid and water-levels in the marine mullet are not significant, but are included in Figures 2 and 4 to facilitate comparison.

The differences between the estuarine and marine *L. richardsoni* are clear. The

Table 1

Levels of body constituents (% of total weight) and energy, E (kJ per gram dry weight) in *Liza richardsoni* from the Bot River estuary and False Bay. Mean values, standard deviations (SD), and numbers of determinations (N)

Size-Class (mm)	Protein (%)	SD	N	Lipid (%)	SD	N	Water (%)	SD	N	Ash (%)	SD	N	E (kJ g^{-1}dw)	SD	N	Total
Bot River																
61	18,00		1	3,04		1	72,87	0,49	2	3,71	0,04	3	14,97	0,23	2	97,62
61–120	17,29	0,47	3	2,75	0,15	2	76,13	1,46	2	3,78	0,88	3	14,50	0,62	2	99,95
121–180	18,07	0,14	3	8,11	0,03	2	70,35	0,50	2	3,19	0,54	3	17,43	0,21	3	99,72
181–240	18,77	0,11	2	8,17	0,63	4	69,40	0,30	2	3,39	0,29	3	18,50	0,21	4	99,73
241–300	17,31	0,15	3	16,68	0,85	3	60,60	1,41	2	3,13	0,27	3	21,15	0,22	4	97,72
301–360	17,35	0,11	3	16,86	0,31	3	61,62	2,41	2	3,60	0,21	3	21,50	0,64	4	99,43
361–420	18,77	0,06	2	14,01	0,20	4	63,17	0,91	2	3,09	0,44	3	20,43	0,33	4	99,04
Mean	17,94	0,65		9,95	6,00		67,73	5,99		3,41	0,29		18,35	2,86		
False Bay																
61	17,58	0,25	3	2,68	0,03	3	75,55	0,14	2	4,73	0,66	3	13,80	0,61	3	100,54
61–120	17,49	0,14	3	1,79	0,05	3	75,84	0,01	2	5,37	0,78	3	13,95	0,81	4	100,49
121–180	19,25	0,26	3	2,11	0,12	3	74,19	0,22	2	4,50	0,33	3	14,32	0,17	4	100,05
181–240	19,08	0,20	3	1,78	0,02	3	74,54	0,92	2	4,81	0,67	3	14,17	0,43	4	100,21
241–300	19,23	0,26	3	2,80	0,09	3	74,24	0,47	2	4,63	0,17	3	14,66	0,36	4	100,90
Mean	18,53	0,91		2,23	0,48		74,87	0,77		4,81	0,33		14,18	0,33		

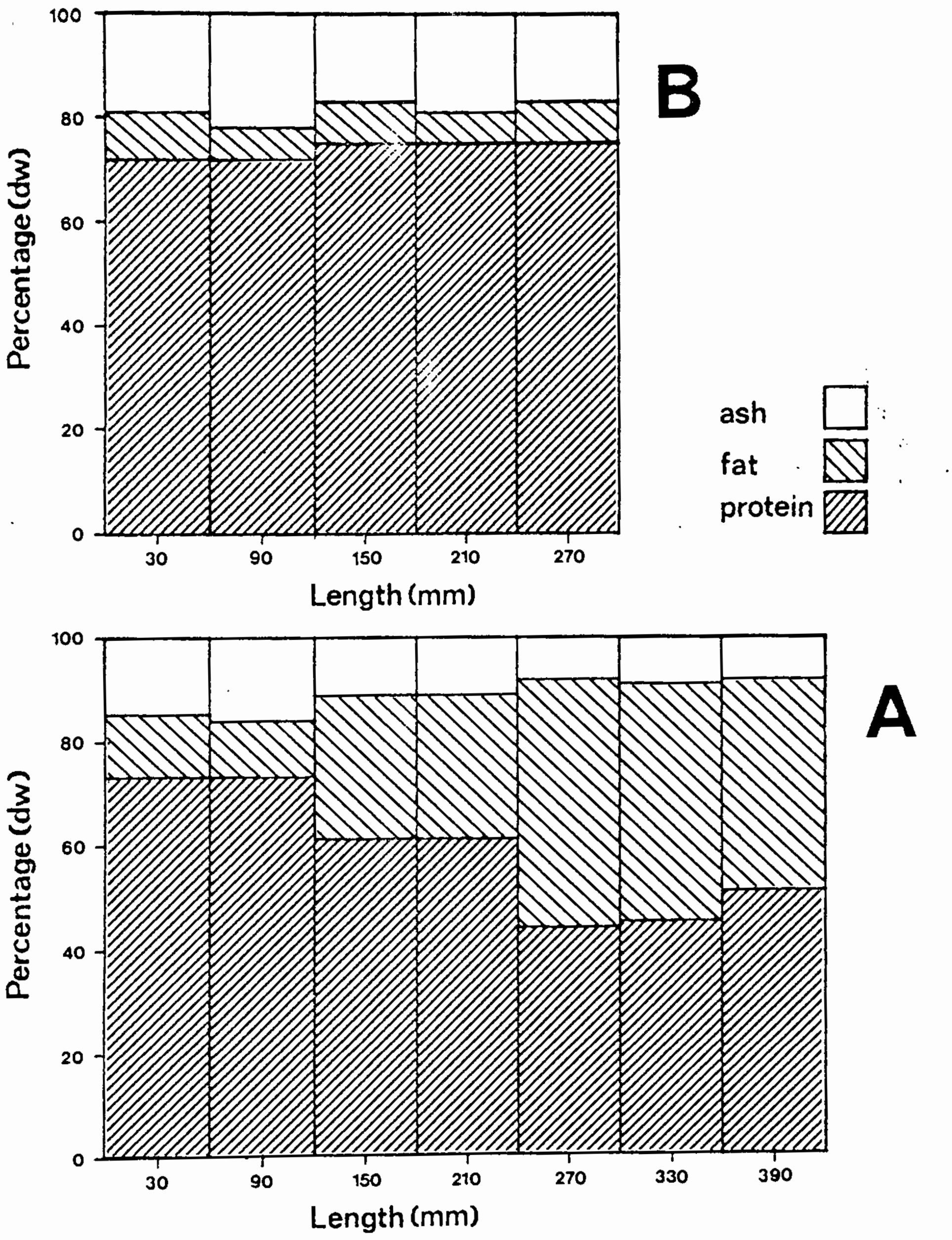

Fig. 1. Changes of body constituents with length in the various size classes of *Liza richardsoni* in the Bot River estuary (A) and False Bay (B), expressed as percentage of the dry weight.

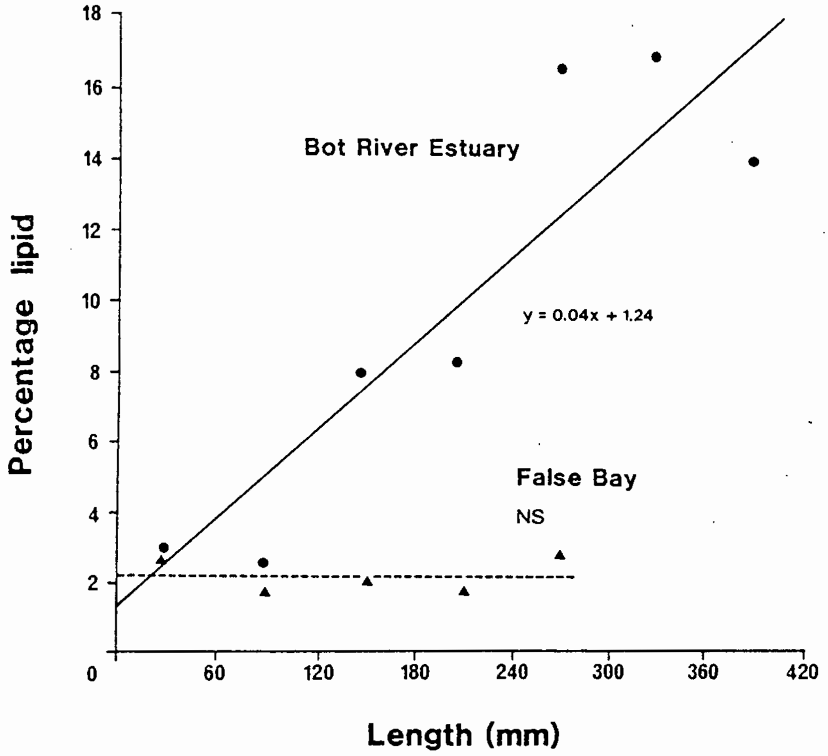

Fig. 2. The relationships between length and percentage lipid content in *Liza richardsoni* from the Bot River estuary (●) and False Bay (▲). For the Bot, $r = 0.89$ $p < 0.01$; for False Bay, r is not significant (NS).

large increases in lipid-levels (Fig. 2) and energy-content (Fig. 3) as well as the decrease in water-levels (Fig. 4) with length in the estuarine mullet are not present in the marine mullet. Figures 1 to 4 also illustrate the similarities between estuarine juveniles (TL <120 mm) and marine mullet of all size-classes. This is particularly evident in Fig. 1. It is important to note, however, that estuarine subadults (TL 120–220 mm) have significantly higher lipid and energy-levels than the estuarine juveniles (Lord's Range test, $p < 0.05$), and that these levels are, in turn, significantly higher in the estuarine adults (TL >220) than in the subadults (Lord's Range test, $p < 0.05$).

DISCUSSION

The results of chemical analyses of body constituents of marine and estuarine *L. richardsoni* indicate that larger estuarine mullet are in better condition than their marine conspecifics. Generally, the body-constituent levels given by Marais & Erasmus (1977) for *L. richardsoni* in the Swartkops estuary are similar to those found in the present study for estuarine mullet, while the levels reported by Van Wyk (1944) for muscle fillets of marine *L. richardsoni* correspond to those found for marine mullet.

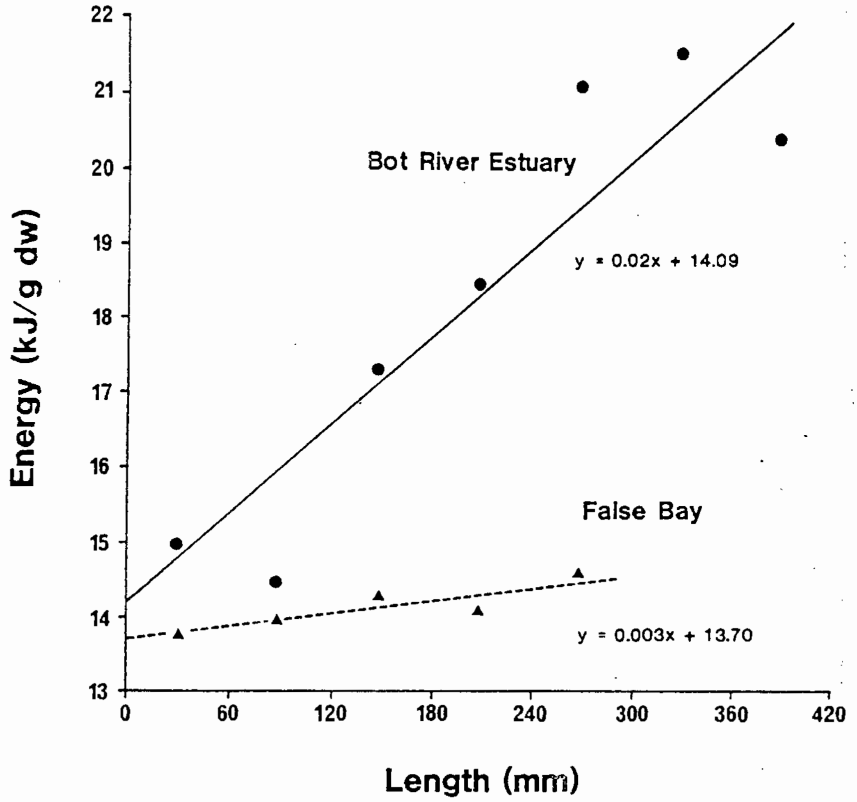

Fig. 3. The relationships between length and energy (kJ g^{-1} dry weight) of *Liza richardsoni* from the Bot River estuary (●) and False Bay (▲). For the Bot, $r = 0{,}92 \pm <0{,}005$; for False Bay $r = 0{,}88$ $p < 0{,}05$.

The migrations of many mullet species in and out of estuaries are well known (Wallace 1975, Wallace & Van der Elst 1975, Blaber & Whitfield 1977, Blaber & Blaber 1980). *Liza richardsoni* spawns in the sea (Lasiak 1983) and the fry may migrate into estuaries, where they remain until adolescence, having to return to the sea to spawn (Blaber 1981, Wallace *et al.* 1984). At the time of sampling for this study, however, the Bot River estuary had been closed for four years (1977–1981). This interrupted the normal migration patterns, preventing the mullet trapped inside from spawning during that period. Recruitment of a limited number of fry and juveniles was possible via the overflow channel to Kleinmond (Bennett *et al.* 1985). The absence of spawning, as well as low recruitment, was evident from the difficulty experienced in catching the smaller size-classes. No ripe and running *L. richardsoni* were caught in the estuary during the same period.

Marais & Erasmus (1977) report that *L. richardsoni* feeds mainly on organic debris, and both planktonic and attached diatoms. Estuaries are therefore ideally suited to mullet, since these food items are present in abundance (Blaber & Whitfield 1977).

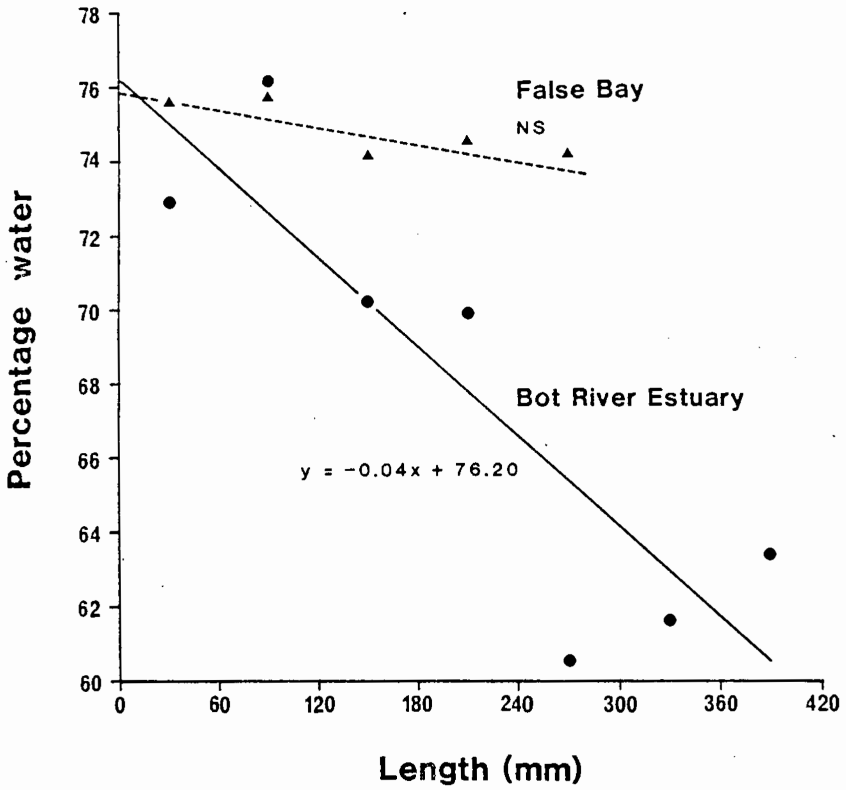

Fig. 4. The relationships between length and percentage water content in *Liza richardsoni* from the Bot River estuary (●) and False Bay (▲). For the Bot, $r = -0{,}87$ $p<0{,}001$; for False Bay r is not significant (NS).

The 'balance of body constituents' of fish is related to their food intake and energy expenditure (Brett *et al.* 1969). Lipid content and condition of *Rhabdosargus holubi*, for example, have been shown to be positively related to feeding level (Blaber 1975). It seems therefore that the higher lipid and energy-levels found in subadult *L. richardsoni* from the Bot River estuary may be attributed to more favourable feeding conditions. Estuarine adults, moreover, had significantly higher lipid and energy-levels than the subadults. Mullet are known to have maximum lipid levels in the pre-spawning season (Deng *et al.* 1976). As spawning never actually takes place inside the estuary, the pre-spawning levels are apparently maintained. It is thus concluded that both the favourable feeding conditions, as well as the absence of spawning, contribute to the higher fat and energy content of the mullet in the Bot River estuary.

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GENERAL CONCLUSIONS

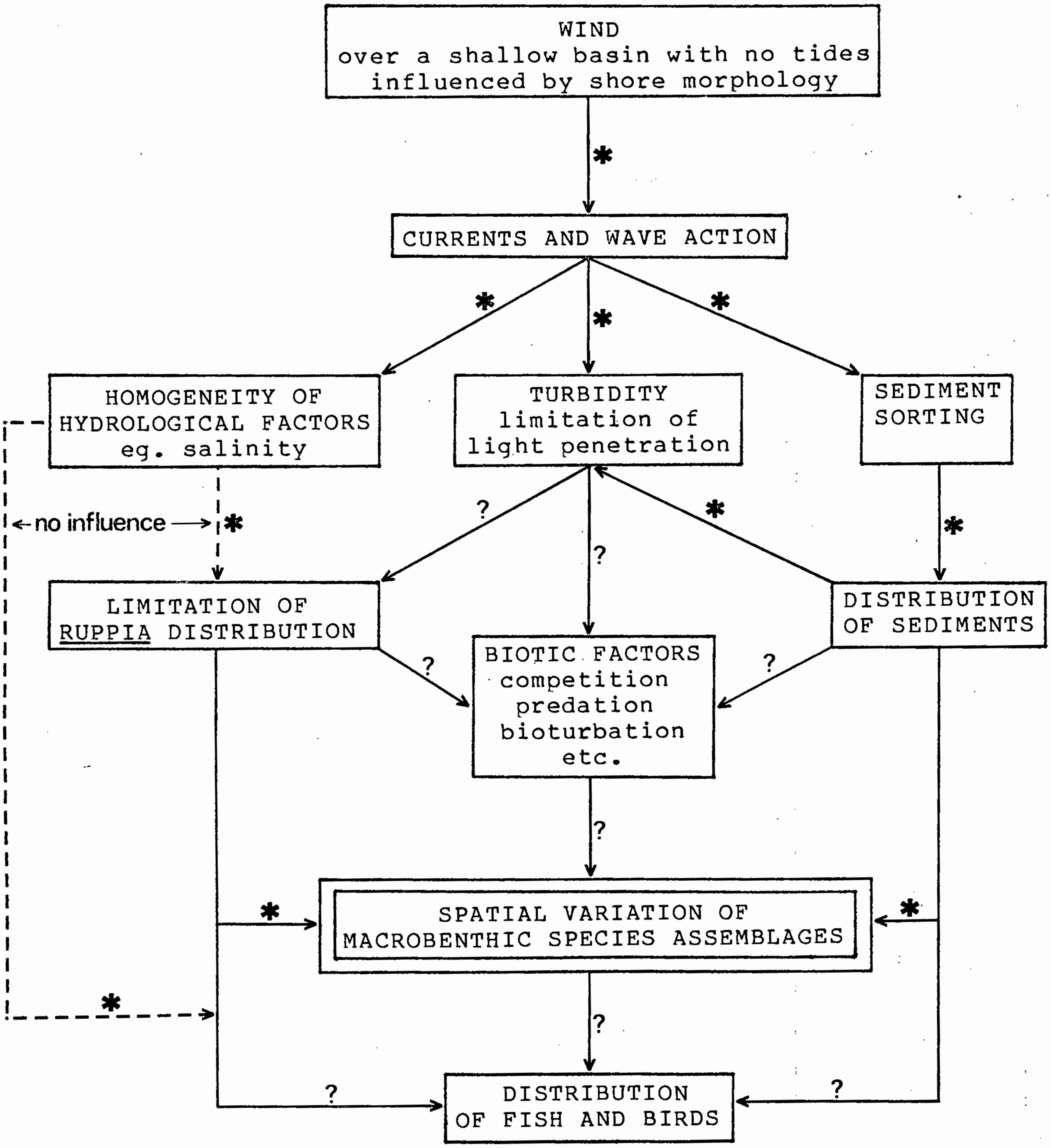
Towards a unified theory

In this section, rather than repeat the conclusions reached in each chapter, an attempt is made to synthesise them into a coherent framework for describing the dynamics of the Bot River estuary, in terms of the interactions between its physical and biological components. This broad framework is represented in Figure 1, which summarises the interactions between environmental factors and the species assemblages whose structure and spatial distribution they control.

The single most sriking feature of the benthic fauna is their poor species diversity. After all the surveys, only 32 species were found, with a maximum of only 27 species in a single survey (May 1983). The low diversities are further emphasised by the fact that only 11 species contributed more than 95% to the total biomass in the estuary. This low species diversity is probably due to two major factors, both related to the sandbar separating the estuary from the sea, but in a paradoxical way. Firstly, the presence of the sandbar prevents the recruitment of new species to the estuary from the sea (Chapter One). As a result, it is becoming faunistically similar to a coastal lake, and if left with the sandbar intact, may develop into one.

Secondly, the removal of the sandbar, on the other hand, causes catastrophic interruption of its development towards a coastal lake and a state of equilibrium, and the system then reverts forcefully to a tidal estuarine condition. Many species cannot survive the accompanying large fluctuations in physical conditions, and high mortalities result (Chapter Two). Episodic

Figure 1: Dynamic interactions of physical factors influencing the spatial variation of macrobenthic species assemblages in the Bot River estuary during periods when it is cut off from the sea. Stars indicate confirmed interactions, while question marks indicate hypothetical ones.



perturbations are known to reduce species diversities and the Bot River estuary serves as a good example. Few species would have the necessarily broad niche dimensions to enable them to survive the environmental conditions typical of both coastal lakes and open estuaries.

Benthic consumers in estuaries require the ability to obtain sufficient food from very refractory resources to ensure survival. The presence of lytic enzymes in the guts of the two dominant invertebrates, *Arcuatula capensis* and *Exosphaeroma hylecoetes*, provides these species with the means, via bacterial pathways, of extracting sufficient nourishment from their environment. Laminarinase, in the digestive system of the bivalve, could possibly be of importance at times of low bacterial biomass, which occurs when salinities are unusually low or high (Chapters Four and Five). This is an example of the biochemical mechanisms which may have had to evolve before these species could colonise the extremely variable estuarine environment.

It is clear from the improved physiological condition of the estuarine *Liza richardsoni*, that richer food resources are available to these fish in the Bot River estuary, compared with the sea (Chapter Six). Although the mullet benefit nutritionally during their residence in the estuary, it is necessary for them to return to the sea in order to spawn. As migration is prevented by the sandbar, these fish are consequently dependent on artificial breachings for this purpose.

The major environmental factor causing invertebrate faunistic structure in most open estuaries is the horizontal and vertical stratification of salinity. Many estuaries have separate steno-, meso- and euryhaline communities which not only increase the species diversity of the system, but also constitute the major faunistic groupings. This heterogeneity of salinity structure is absent in the Bot River estuary, not only due to the absence of tidal influence, but also to frequent, strong winds over the shallow basin. As a result, the water is well-mixed and hydrological factors such as salinity, temperature and dissolved oxygen do not contribute to spatial variations in benthic species composition. It has, nevertheless, been possible to demonstrate that distinct spatial patterns do exist in the community structure of the Bot River estuary. It is true that they may not be distinguishable by very different species assemblages, as they are in open estuaries, but they can, at least, be recognised by large differences in the relative abundances of the dominant species in each community (Chapters One and Three).

While salinity sets an inescapable physiological limit on species distributions, the nature of the substratum and its suitability for particular species is often the second-most important, although less stringent, structuring factor in open estuaries. The sand shrimp, <u>Callianassa kraussi</u>, for example, would have difficulty constructing its delicate burrow systems in substrata of glutinous mud. In open estuaries, sediments are usually sorted and distributed by tidal currents which scour the deeper areas, removing fines and depositing them in mudflats along the shores. In the Bot River estuary, however, this pattern of sediment distribution is reversed when the estuary is closed: the fines are removed from the sediments along the shores by the action of wind-generated waves, and deposited in the central, deeper areas of the estuary. Only once the sandbar has been breached, is a channel with a coarse-grained substratum scoured in the region of the mouth, where current velocities are highest. Tidal currents, however, affect only the southern, seaward part of the estuary, which is also further influenced by the importation and deposition of sand by aeolian transport from the dunes. The resulting sedimentary distribution is one of the most important factors controlling biotic spatial patterns in the Bot River estuary (Chapter Three).

Wave action in the shallow estuary has a further important role to play. By re-suspending the fine sediments from the bottom, the turbulence causes the water in the estuary to be almost permanently turbid. The degree of turbidity varies horizontally and vertically for a variety of reasons. The wind strength and fetch (the distance that the wind blows over the water), are important in determining the wave height and thus the amount of turbulence in the water column. These factors, in turn, are affected by the shore morphology of the estuary. The turbidity is further influenced by the nature of the substratum and is usually highest over muddy, shallow bottoms. Currents, also generated by the wind, transport the turbid water to other parts of the estuary.

By limiting the penetration of light to the deeper water, turbidity controls the colonisation of the major macrophyte, _Ruppia maritima_. The effect of _Ruppia_ distribution on the benthic macrofauna is profound and is another important factor structuring the macrobenthic communities (Chapter Three). It also has an indirect influence on species distributions by its effect on other biotic factors, such as predation, bioturbation, and recruitment. It seems, therefore, that the distribution of the sediments and of _Ruppia maritima_, which are inherently linked to the wind regime and morphology of the estuary, are the two most important determinants of benthic macrofaunal distribution.

Since the distribution of the larger consumers, i.e., fish and birds, is influenced by that of their prey, they have been included in Figure 1. A dynamic model such as this is useful for the generation of testable hypotheses and for highlighting gaps in our knowledge. Arrows with asterisks indicate confirmed interactions, while those with question marks are hypothetical and warrant further investigation.

Although the work in this thesis has been directed primarily towards an understanding of the benthic macrofauna, at the conclusion it is not only an insight into the structure of the macrobenthos which has been gained - the dynamic functioning of the whole system has also been elucidated, yielding results relevant to the design of a management plan for the Bot River estuary (see Supporting Paper).

Cape Town, 18 January 1986.

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And then there began to be parlous times in the land of the ecologists, for man made the environment to be sick, and some said there was no redress from the scourge. There arose a cry in the land for relevant research to cure the sickness. Yet those that understood the message would also know that beyond relevant research is the further and greater goal of research to find what is relevant, and the only hope for our world is to continue, as we have been taught, to seek unfettered truth.

Gordon Riley

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SUPPORTING PAPER

Synopsis of the impact of artificially opening the mouth of the Bot River estuary: implications for management

by

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SYNOPSIS OF THE IMPACT OF ARTIFICIALLY OPENING THE MOUTH OF THE BOT RIVER ESTUARY: IMPLICATIONS FOR MANAGEMENT

By

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SUMMARY

This paper synthesizes previous research on the Bot River estuary, including that on its past and present geology, sedimentology, hydrology, history, plant life, invertebrates, birds and fish. The estuary is periodically breached to form a deep mouth and this restores estuarine conditions and allows recruitment of fish; but it also disrupts the system and results in massive losses of aquatic weeds and invertebrates, and a twentyfold reduction in bird-life.

A key feature in the ecology is that the Bot is connected to the adjacent Kleinmond estuary by overflow channels which act as a spillway when water-levels in the Bot are high. Because of this the Bot will slowly become diluted by river-water and change into a fresh-water lake if it is not breached.

Six proposed plans for the management of the Bot River estuary are discussed. Only two are viable: either the estuary should never be breached (and thus be managed as fresh-water lake) or breaching should be controlled and undertaken about every three or four years or when salinities drop below 6‰ (and the system managed as an estuary).

INTRODUCTION

Of all ecosystems in southern Africa, estuaries are probably the most threatened by human activities. Compared with the situation in many other parts of the world, they occupy a relatively small area of the coastline and they are few in number and small in size (Day 1981). The primary reason for this is the low and strongly seasonal rainfall experienced over much of southern Africa. many of the estuaries are open for part of the year only, so that they are especially vulnerable to activities such as dam-building and irrigation which reduce river-flow (Begg 1978, Heydorn & Tinley 1980). In particular, their importance as nursery grounds for juvenile fish (Day *et al.* 1981, Blaber 1981) has recently been analysed in relation to the potential effect of further estuarine degradation on fish stocks (Wallace *et al.* 1984).

The Bot River estuary (34°20'S 19°06'E) lies on the south-western coast of South Africa and is the third largest estuary in the Cape Province. It is an important wetland for a variety of birds, being one of the ten most significant coastal wetlands for waders in the south-western Cape (Cooper *et al.* 1976, Underhill & Cooper 1982). It easily qualifies as a wetland of international significance, as recognized by the Ramsar convention (Smart 1976), since it supports up to 36 000 red-knobbed coot *Fulicia cristata* as well as significant numbers of various species listed in the Red Data Book (Siegfried *et al.* 1976) as being rare or endangered: the greater flamingo *Phoenicopterus ruber*, lesser flamingo *P. minor*, Cape shoveller *Anas smithii*, Caspian tern *Hydro-*

Trans. Roy. Soc. S. Afr., 45, Parts 3 and 4, July, 1985.

progne caspia and white pelican *Pelecanus onocrotalus* (Siegfried 1977, Siegfried *et al.* 1976, Heÿl & Currie 1985).

The estuary also contains large numbers of fish. Thirty-two species have been recorded there, including four which are dependent on estuaries throughout their lives and six which rely on estuaries as nursery grounds (Bennett *et al.* 1985).

A survey has shown that 90% of the people who visit the estuary do so to fish (R. Bally unpubl. data, Stern 1983), and commercial netting has been permitted at times. The estuary is also used for yachting, boating, swimming and vacations; recreational shooting of waterfowl takes place on private land on the Lamloch swamps. These uses of the estuary have to be considered together with the need for conservation when drawing up management plans for the area.

The Bot River estuary is normally closed to the sea, its mouth being blocked by a wave-formed sandbar. But for over 80 years the mouth has been artificially breached every few years, initially to restore populations of marine fish and thus improve the fishing, and more recently to prevent flood-waters from threatening properties on the banks. This action has major repercussions on the ecology of the estuary and was the central issue that prompted a research project on the estuary which began in 1981. Although initially led by the Departments of Zoology and Geochemistry at the University of Cape Town, and funded by the South African National Committee for Oceanographic Research, the research soon attracted workers from other institutes and eventually 19 researchers from nine different departments became involved. Most of their work is reported in this volume—the proceedings of a symposium on the estuary—and in earlier summaries by Koop (1982) and Koop *et al.* (1983). The purpose of this paper is to synthesize this work; to outline the consequences of opening the mouth artificially, documenting, in particular, the breachings of 11 August 1981, 10 October 1981 and 29 June 1983; and to relate these findings to possible management options.

DESCRIPTION

The Bot River estuary has a catchment of about 900 km^2 and the estuary itself covers an area of between 9 km^2 and 23 km^2, depending on the depth of water. The mouth is closed almost all the time, but appreciable variation in the salinity occurs due to the effects of the sea, so that following Day's (1981) definition it can be considered a closed estuary.

The bathymetry is described by Willis (1985). Briefly, the mean depth is 1,5 m below mean sea-level (MSL), the deepest section, which lies close to the south-east bank, being 2,5 m below MSL (Fig. 1).

The nature of the sediments is closely linked to the bathymetry. The marginal areas consist of a non-cohesive sandy sediment, while in the deeper areas a cohesive sediment, more than 90% of which is mud, accumulates (Willis 1985). Wind-induced currents are greater around the margin of the lagoon (Van Foreest 1985) and winnow out finer particles which deposit in the deeper regions. The shallowness of the estuary also results in considerable mixing of the water, and physical conditions are

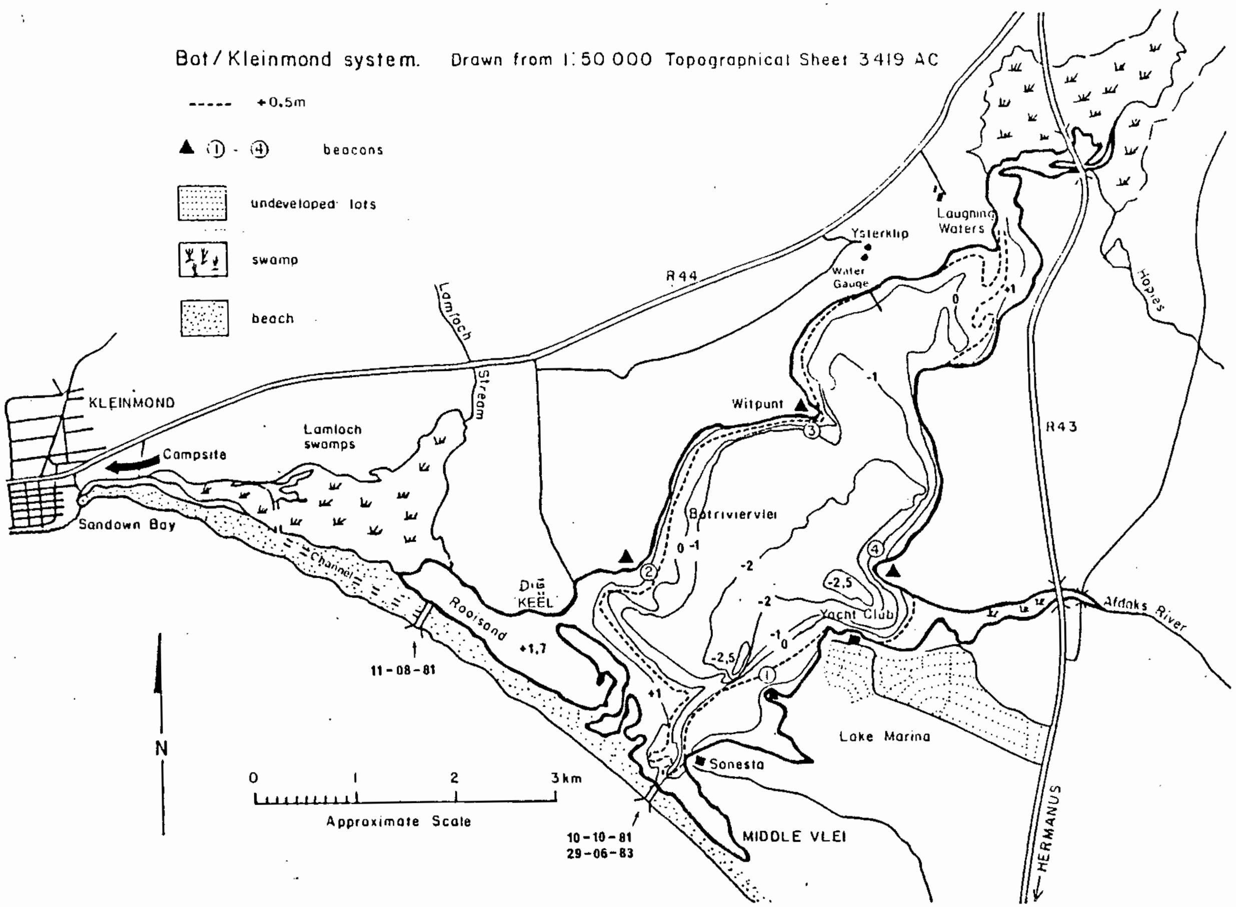

Fig. 1. Map of Bot River estuary, showing the bathymetry and locations of Rooisand, the overflow channel and the connections with Kleinmond estuary via Lamloch swamps. Also shown are the positions (and dates) of breachings.

comparatively uniform throughout the lagoon. Wind-driven waves continually disturb the sediments so that the water is usually muddy and turbidity high. Periodic floods deposit massive amounts of sediment. For example, Willis (1985) records that after the floods of January 1981, a layer of 12 cm thick (equivalent to 85 kg dry sediment^{-2}) was deposited in the upper third of the lagoon.

Water-level varies seasonally and from year to year (Fig. 2) rising as high as 2,8 m above MSL (when the maximum depth is about 5,3 m) and falling to 0,4 m above MSL when the mouth is breached. In 'normal' years, such as 1979 to 1980, when the mouth is left closed, the estuary fills during the winter rains (usually June to August), and then declines during summer and autumn due to evaporation. Unseasonable rainfall may, however, fill the estuary earlier than normal (e.g. January 1981).

Salinity also varies seasonally being highest in summer and autumn, declining in winter due to dilution by river-water. Superimposed on this pattern there is a progressive reduction in salinity from year to year if the mouth remains closed (see 1978 to 1981, Fig. 2). This is a key feature, for it means that the estuary will become a freshwater lake if left permanently closed. Nutrients, on the other hand, vary comparatively little from year to year, riverine levels of phosphate, nitrate and silicon equalling or exceeding those in the estuary and thus replenishing any losses that occur (Bally & McQuaid 1985).

Near the coast the estuary has a shallow western arm—Rooisand— which has an average depth of 1,7 m above MSL and which is connected to the tiny Kleinmond estuary via the Lamloch swamps. In addition, an overflow channel between Rooisand and Kleinmond estuary runs in the dune slacks (Fig. 1). Rooisand plays a vital role because water flows through it from the Bot to the Kleinmond estuary (and thence out to sea) whenever levels in the Bot exceed approximately 2,0 m above MSL. Thus the

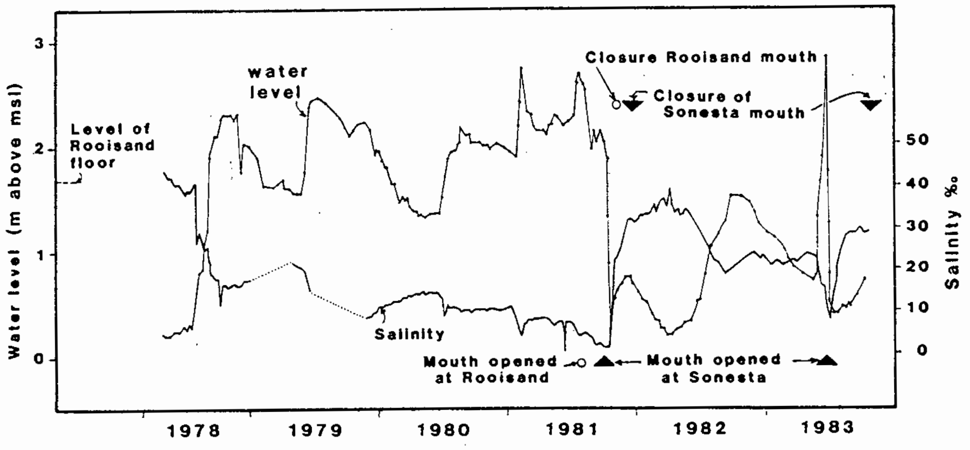

Fig. 2. Water-levels and salinity in Bot River estuary, 1978 to 1983. Dates when the mouth was breached at Rooisand and at Sonesta, and subsequent closures of the mouth, are indicated by circles (for Rooisand) and triangles (for Sonesta).

water in the Bot cannot rise much above 2,8 m above MSL—the maximum recorded so far—since Rooisand acts as a natural spillway. Thus it is unlikely that the Bot will achieve a sufficient head of water to break open its mouth naturally. It also means that the estuary is gradually flushed by successive winter rains, and from year to year the salts are diluted. Willis (1985) has measured the rate of water-flow through the overflow channel and recorded a flow of 0,5 m s^{-1}, equivalent to a loss of 310 000 m^3 d^{-1} or a drop in water-level of 11 cm per week in the Bot (assuming no further input).

To reiterate, the overflow channel and the connections between Rooisand and Kleinmond are of central importance, since they are the cause of progressive dilution of the Bot.

HISTORICAL CHANGES

Massive long-term changes are known to have occurred in the Bot estuary. The system is a relict valley and has varied from a completely closed coastal lagoon to a massive estuary with a wide-open mouth. These changes reflect regressions and transgressions of the sea as it has risen and fallen by as much as 500 m during successive glacial and post-glacial periods. At present the estuary is reaching the end-stage of its life as an estuary, the bar across the mouth cutting it off from the sea (Rogers 1985).

An early newspaper report of 1838 describes the estuary as a port, suggesting that in recent times it was freely open to the sea. However, Bally (1985) has reviewed all the evidence and concludes that the Bot could not have been used as a harbour at that time since its mouth would have been closed most of the time. Bally also describes large dune-fields which covered the present Rooisand area between 1880 and 1903 and must have restricted or prevented flow between the Bot and Kleinmond estuaries. Indeed, there is evidence that the connection between the two systems increased between 1884 and 1960. Van Heerden (1985) argues that Rooisand is a gradually sinking deflation area, suggesting that the connection between the estuaries will increase further. However, the floor of Rooisand is made of a hard peaty layer which resists scour (Rogers 1985), so that water passing between the two estuaries will only slowly cut through Rooisand: its relatively shallow floor is thus likely to remain a barrier except when water-levels in the Bot exceed the height of Rooisand, i.e. 1,7 m above MSL.

ECOLOGY

The growth of plants in the estuary has been measured by Bally *et al.* (1985) who estimated a production of 13 000 tonnes (dry weight) per year. The aquatic weed *Ruppia maritima* is of greatest importance and, together with three other submerged weeds, *Potamogeton pectinatus*, *Chara* sp. and *Cladophora* sp., contributes 72 % of the production. Benthic diatoms (7 %), phytoplankton (7 %) and reed-beds (14 %) make up the balance. *Chara*, the reeds and *Potamogeton*, being limited to regions of low salinity, are concentrated at the head of the estuary. *Ruppia* dominates most of the estuary (Fig. 3) but is restricted to water-depths of less than 2,5–3,0 m because turbidity limits light penetration. *Potamogeton* is restricted to the shallows of Swartvlei

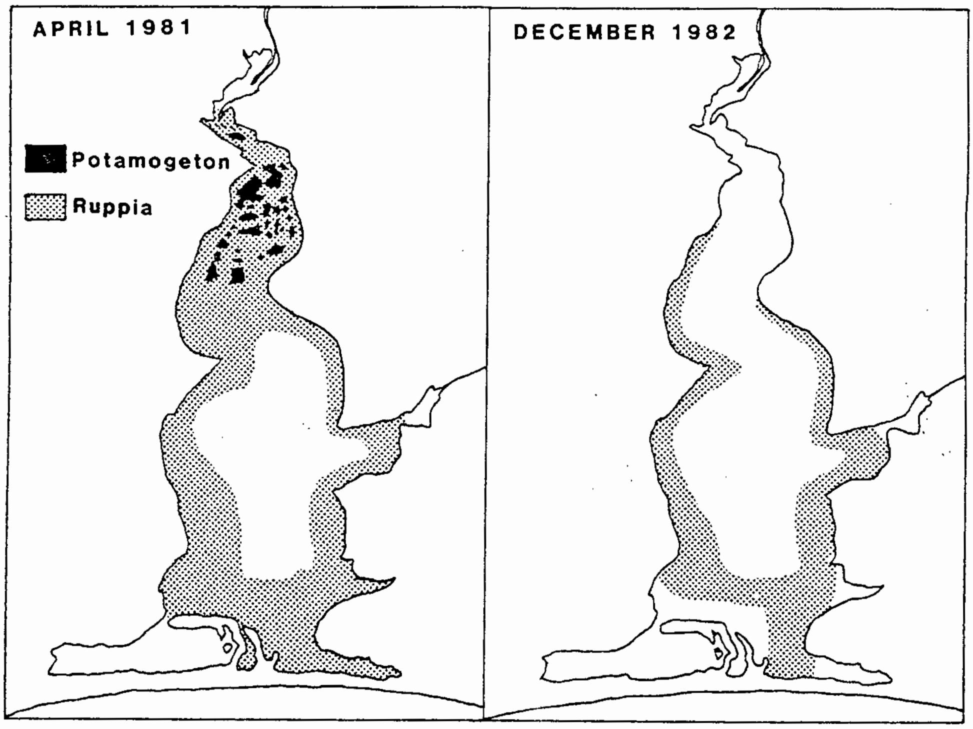

Fig. 3. Distribution of the weeds *Potamogeton pectinatus* and *Ruppia maritima* in April 1981, prior to breaching of the mouth, and in December 1982, 14 months after these weeds had been largely eliminated by a breaching of the mouth that took place in October 1981.

lake in a comparable manner by darkly stained 'blackwater' (Howard Williams & Liptrot 1980).

The weed-beds have an annual cycle of growth, and bacteria play a major role in their decomposition. Roberts (1984) and Roberts *et al.* (1985a, b) discuss how particular bacteria attach directly on to *Ruppia*, some of which initiate degradation of the still-living plants. A second group of bacteria occurs in the water column and depends on suspended organic particles. These free-floating bacteria have average densities of 2×10^6 cells ml^{-1}, peaking in summer. Drastic reductions of their numbers occur when the mouth is breached although they recover within weeks. Tests on the tolerances of these free-floating bacteria show that they grow best in 50% sea-water, moderately in 100–200% sea-water, but very poorly in 10% sea-water. Catastrophic changes in salinity following flooding or breaching of the mouth are thus likely to have radical effects on the bacteria. Their growth is also retarded at low temperatures, possibly explaining the reduction of bacteria in winter. A third group of bacteria—the anaerobic sulphur bacteria—occur beneath the surface of the sediment, particularly where cast-up mats of *Ruppia* accumulate, and are probably of major importance in the decay of *Ruppia* after its summer die-back.

The benthic invertebrates are characterized by an extremely low diversity, only 25 species having been recorded (De Decker & Bally 1985), and the average biomass (6,3 g dry weight m^{-2}) is relatively low. Only six species are consistently abundant, and they account for about 95% of the biomass. Notable is the small mussel *Arcuatula capensis* which contributes 75% of the biomass; although it is likely that the sand-prawn *Callianassa kraussi* (which has not yet been adequately sampled due to its deep-burrowing habit) is also important. Many of the species typically occur in low-salinity habitats and are often present in coastal lakes.

The benthic invertebrates can be divided into four groups, respectively associated with the littoral zone at the top of the shore, the submerged bare sand zone below this, the weed-dominated shallows, and the deep areas. As examples, the sand-prawn *C. kraussi* occurs largely in the shallow sandy areas and the mussel *A. capensis* is almost entirely associated with the weed-beds. A striking feature is that most of the invertebrate biomass (80%) is concentrated in the weed-beds (Fig. 4).

The zooplankton (Coetzee 1985) and meiofauna (De Decker & Bally 1985) are also very poor in species. The zooplankton is dominated by a single species, the copepod *Pseudodiaptomus hessei*, which constitutes 80% of the numbers. The zooplankton appears to be adversely affected by low salinities, declining when the estuary is diluted. The biomass of the zooplankton is low (mean 53 mg m^{-3}) and the numbers of meiofaunal organisms are the lowest recorded for any estuary in South Africa, averaging 0,35 animals cm^{-3}.

Taken collectively, the unusually low diversity of all three invertebrate groups reflects the instability of the system. Irregular opening of the mouth precludes either an estuarine or a freshwater community from becoming fully established.

Large numbers of fish occur in the estuary (Bennett *et al.* 1985) although only 32

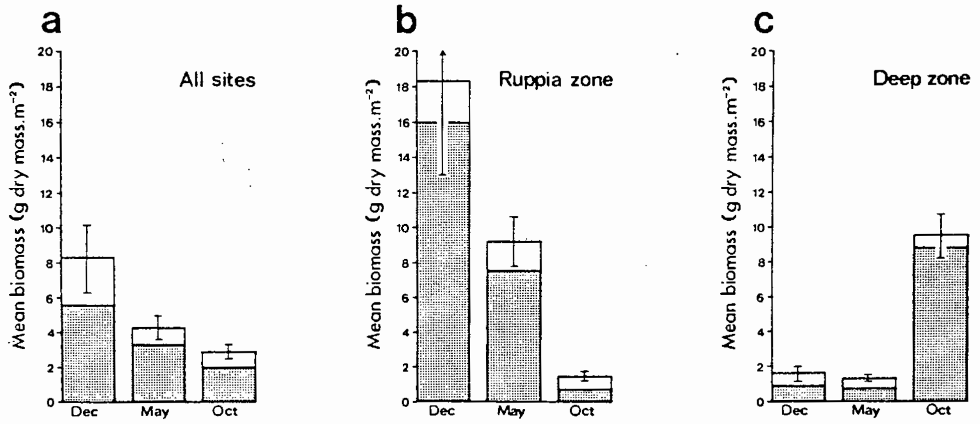

Fig. 4. The mean biomass (g dry mass m^{-2} ± S.E.) of benthic invertebrates in December 1982 and May 1983, prior to breaching of the mouth, and in October 1983, after the mouth had been breached: *(a)* mean figures for all sites; *(b)* within the *Ruppia* zone; *(c)* within the deep zone below the *Ruppia*. Shaded areas indicate the contribution of *Arcuatula capensis*.

species have been recorded (Table 1). Amongst these are several species of great importance to anglers, such as the white steenbras *Lithognathus lithognathus*, leervis *Lichia amia*, and elf *Pomatomus saltatrix*. Virtually all of the fish are estuarine or marine, including four small species which are restricted to estuaries and six larger species whose juveniles are dependent on estuaries (Wallace *et al.* 1984). Species in this latter group breed at sea, but estuaries serve as nursery grounds for their juveniles. Among them are the white steenbras *L. lithognathus*, Cape stumpnose *Rhabdosargus holubi*, leervis *L. amia* and flathead mullet *Mugil cephalus*. De Decker and Bennett (1985) have shown that the condition of one of the species that is regularly found in estuaries, the southern mullet *Liza richardsoni*, is better in estuaries than in the open sea. Estuarine specimens have a higher fat and energy content (9,9 % fat, 18,35 kJ g^{-1}) than marine specimens (2,2 % fat, 14,18 kJ g^{-1}), so it appears that there is more food available to fish in estuaries than in the sea.

Of the marine fish recorded in the Bot, almost all depend on a deep, tidal mouth being breached at Sonesta (Fig. 1) if they are to recruit into the estuary. Only the southern mullet *L. richardsoni* seems able to recruit in any numbers via the overflow channels that link the Bot with Kleinmond estuary (Bennett *et al.* 1985).

The Bot River estuary is an important habitat for water-birds and they play an integral role in the system, as they do in many southern African estuaries (Siegfried 1981). Notable are large numbers of the red-knobbed coot *Fulicia cristata* (up to 36 000) and over 1 000 each of the yellow-billed duck *Anas undulata* and the southern pochard *Netta erythrophthalma*, but a further seven species of Anatidae (ducks and geese) have also been recorded (Heÿl & Currie 1985).

The highest biomass recorded for all the birds combined exceeds 40 000 kg. By far the largest contributors are herbivorous birds (principally the red-knobbed coot) which total as much as 38 000 kg (Fig. 5). In terms of biomass the greater flamingo is the most important of the invertebrate-feeders, although waders are numerically more abundant. the fish-eating birds are dominated by the reed cormorant *Phalacrocorax africanus*, but even considered collectively the fish-eaters contribute little to the avian biomass (Fig. 5).

Because of its abundance, a particular study has been made of the red-knobbed coot, which shows that it eats up to 759 tonnes (dry weight) of the weed *Ruppia* each year, returning 470 tonnes of faeces to the system (Stewart & Bally 1985).

A preliminary model of the flow of carbon through the estuary has been drawn up by Roberts (1984), which emphasizes the central roles played by the weed *Ruppia*, the bacteria, the red-knobbed coot and the fish.

THE CONSEQUENCES OF ARTIFICIAL BREACHING

Physical characteristics

The Bot River estuary is normally breached at Sonesta, creating a deep, tidal mouth which remains open for between two and four months. Immediately after breaching, the outflow of water is massive and creates a mouth that is between 80 and 110 m wide, 2,0–2,5 m deep, with a cross-sectional area of 106–165 m^2. Flow rate

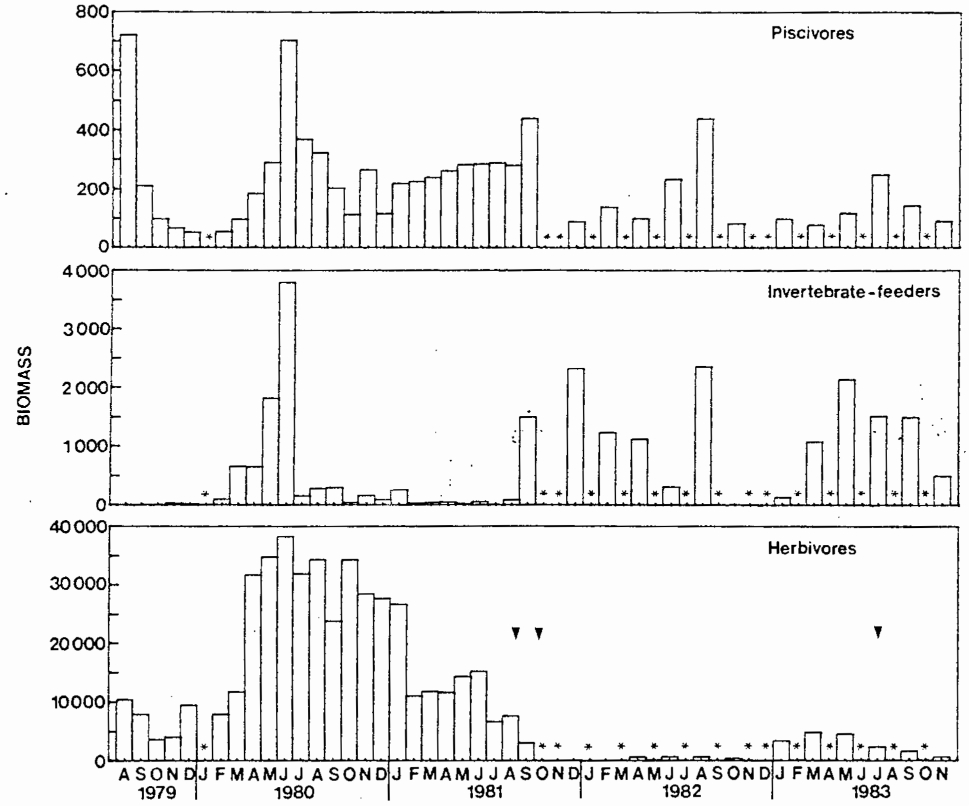

Fig. 5. Biomass values (kg) for fish-eating, invertebrate-feeding and herbivorous birds on Bot River estuary for the period August 1979 to November 1983. Asterisks indicate absence of data, and arrows show when breaching occurred.

reaches 2.48 m s^{-1}. Following the breaching of 10 October 1981, the initial discharge was 254 m^3s^{-1}, but after the breaching of 29 June 1983 (when the estuary was fuller) it reached 409 m^3s^{-1} (Fromme 1985).

Water-level in the estuary normally changes relatively slowly, but it can change abruptly following floods as, for example, in January 1981 when the volume of the estuary increased by 50% in a few days (Fig. 2). Even more dramatic changes follow breaching of the mouth at Sonesta, water-level declining from as much as 2.8 m above MSL to 0.4 m above MSL in 24 h (Fig. 2, Willis 1985). The decrease in water-area can be as much as 60%—from 23 km^2 to 9 km^2.

After the initial outflow the estuary stabilizes at a level of about 0.4 m above MSL and becomes tidal, although the tidal rise and fall is only 15 cm. The resulting penetration of sea-water raises salinities to almost 35‰ throughout the body of the lagoon. Once the mouth closes, salinity normally declines slowly, although for brief periods it may rise due to evaporation, and hypersaline conditions have been recorded (Bally & McQuaid 1985, Willis 1985).

Tidal currents at the mouth are flood-dominated, introducing marine sand into the estuary. This is reflected in the high percentage of carbonate in the sediments near the mouth (Willis 1985). On the other hand, the initial outflow of water that follows breaching scours a deep channel, exporting sediment, and fine sediments which are suspended by wind action are flushed out. The clarity of the water rises abruptly as the estuarine water is replaced by clear sea-water and the increase in the salt content may encourage flocculation of fine particles, further increasing water clarity.

Cutting a mouth at Sonesta severs any links between the Bot and Kleinmond estuaries since the water-level drops below that of Rooisand. The reduction of flow of water from the Bot to Kleinmond increases the chance that the mouth of the Kleinmond estuary will close.

Because of the massive outflow that follows breaching at Sonesta, and because of the huge impact of this on the ecology (see below), the estuary was experimentally breached at Rooisand on 11 October 1981. The aim of this was to restrict the outflow and the decline in water-level, since the floor of Rooisand lies at 1,7 m above MSL and thus sets a limit on how far the water-level can drop. This objective was achieved, the level dropping to only 1,9 m above MSL before stabilizing (Fig. 2). The escaping waters scoured a mouth 50 m wide, 0,48–0,97 m deep and 30 m^2 in cross-section. Maximum discharge was only 70 m^3s^{-1}, substantially less than at Sonesta, primarily because the hydrostatic head at Rooisand was only 1,0 m while that at Sonesta was 3,0–4,3 m (Fromme 1985). However, the Rooisand breaching prevented tidal penetration into the estuary, Rooisand being above the height of even high-water spring tides. Thus the salinity in the Bot dropped with increasing rapidity as water flushed through the estuary and out of the Rooisand mouth, exaggerating the normal overflow into Kleinmond estuary.

The key differences between the two breaching sites therefore lie in the relative amounts of water loss and in their contrasting effects on salinity.

Biological characteristics

Breaching the estuary at Sonesta flushes out large amounts of water-weed and leaves the remaining weed-beds stranded above water. Virtually all the *Ruppia* (which contributes 72% of the primary production) is eliminated, because this weed is confined to shallow areas. Recovery is due to germination of seeds, and is slow. Figure 3 shows that the area covered by *Ruppia* in December 1982, 14 months after the previous breaching, was only 40% of that recorded in April 1981, after prolonged closure of the estuary. *Potamogeton*, restricted to the head of the estuary in April 1981, was still totally absent in December 1982 because higher salinities prevailed after the breaching at Sonesta in October 1981.

A rather less obvious effect of water-level on primary production is the influence on the area that is shallow enough for the growth of aquatic weeds. If the area potentially available for their growth is plotted against water-level, the relationship is not a simple one, but depends on the shape of the estuarine basin. Between water-levels of 2,0 and 1,0 m above MSL, the available area changes little, but a further drop in water-level to 0,5 m results in a decline of 10 % in the area that can be colonized (Bally *et al.* 1985).

The least direct effect is that involving a time factor. The speed with which the estuary refills after the mouth closes will determine how fast new areas are inundated and can be colonized, and the rate at which deeper areas become too deep for continued growth of weeds. Because there is a lag of between 2 and 6 months before *Ruppia* begins to grow in newly submerged areas, the speed at which water-levels change can be critical.

Breaching the mouth at Rooisand reduced the loss of weeds because the drop in water-level is smaller. But it does nothing to check the progressive reduction in salinity, so that the estuary will become a fresh-water lake in the absence of breaching at Sonesta. Some of the potential changes to the aquatic flora that would ensue are outlined later in this paper.

Changes in the bacterial populations of the Bot do occur after breaching at Sonesta, the rise in salinity reducing bacterial numbers; but this is a short-term effect soon countered by the adaptability of the diverse bacterial community.

Dramatic declines in the benthic invertebrates follow breaching at Sonesta. Since most of the invertebrates are associated with *Ruppia*, reduction of this weed automatically depletes these invertebrates. Figure 4 shows that after the breaching of June 1983, the benthic biomass within *Ruppia* beds was reduced by between 84 and 92%; although in the deeper zones it rose. Overall, biomass declined 54%. Davies (1982) has documented a comparable decline in invertebrate biomass following a regression of aquatic weeds in Swartvlei Lake.

If the Bot River estuary is left to become a fresh-water lake, the invertebrate fauna will change substantially, brack-water and estuarine species giving way to fresh-water insects. In particular, the sand-prawn *Callianassa kraussi* will be unable to breed, since it cannot reproduce in salinities below 17% even though adults survive in very low salinities (Forbes 1978). It is notable that the only post-larval stages of *C. kraussi* recorded from the Bot were collected shortly after October 1981, when breaching at Sonesta had raised salinities (Coetzee 1985). Also interesting is the fact that *C. kraussi*, whose burrows are left exposed by the falling water-level after a breaching, remains alive for several months and can begin recreating its burrows immediately it is inundated again. After an exposure period of 10 months at one site, burrow densities of 21,7 holes m^{-2} were recorded immediately after inundation (Bally, unpubl. data).

The number of birds using Bot River estuary declines drastically after the mouth is breached. Their biomass dropped from a peak of about 40 000 kg to 700 kg after the two breachings in August and October 1981 (Fig. 5). This pattern was most obvious for the herbivorous birds, and could be directly related to the almost total elimination of *Ruppia*, their major food plant. Recovery of the herbivorous species is also slow. Their biomass remained low throughout 1982, began to increase in 1983, but declined again when the mouth was opened once more in June 1983 (Fig. 5). Heÿl and Currie (1985) estimate that it takes two to three years after a breaching for the birds to re-establish their numbers.

The numbers of fish-eating birds also diminish after a breaching at Sonesta, although their response is less marked than it is for herbivorous species. Invertebrate-feeders, on the other hand, increase in numbers and biomass when the water-level drops. This is presumably because they are dominated by the greater flamingo which can feed only in very shallow water, and a lowering of the water-level exposes previously inaccessible areas where they can feed (Heÿl & Currie 1985).

If the estuary were to be breached at Rooisand in the future, the effect on the birds would be reduced. The water-level would not drop as suddenly or to such an extent. The increasing dilution of the water would result in *Potamogeton* replacing *Ruppia*. But since *Potamogeton* is readily eaten by red-knobbed coot, this change would probably have little effect on the birdlife.

Twenty-nine of the thirty-two fish species in the Bot are marine or estuarine species which are unlikely to survive fresh-water conditions (Table 1). Opening the mouth at Sonesta restores salinities and allows recruitment of these species. Some of them are detritus-feeders and must, ultimately, depend on *Ruppia* as a source of detritus. Others which are invertebrate-feeders depend indirectly on *Ruppia* since by far the largest biomass of invertebrates is associated with the weed. When the estuary is closed for prolonged periods *Ruppia* increases, but its total collapse after breaching of the estuary may be detrimental to the fish. Certainly the regression of weed-beds that took place in Swartvlei Lake in 1979 was associated with a 54% decline in the numbers of two dominant fishes, and a decrease in their 'condition' (Whitfield 1984).

Opening the mouth of the Bot at Rooisand accelerates the decline in salinity, and Bennett (1985) recorded a mass mortality of fish in October 1981, following the Rooisand breaching on 11 August 1981, when the salinity dropped to 3–4‰. For nine of the estuarine species present in the Bot, lethally low salinities were probably reached prior to the mass mortality, at about 5–8‰. A further nine species died during the mass mortality while the remaining 11 species appeared to survive salinities as low as 3‰ (Table 1). During this time three fresh-water species made their appearance in the estuary, notably the exotics *Cyprinus carpio* (carp) and *Micropterus salmoides* (bass) (Table 1).

MANAGEMENT OPTIONS

The Bot River estuary is used for a variety of purposes by a variety of users, and no management plan will ever satisfy all needs. At various times six different suggestions have been made for its management. 1. Discontinue artificial breachings. 2. Construct a weir at the mouth. 3. Raise and fix the dunes at the mouth. 4. Continue *ad hoc* breachings at Sonesta whenever water levels rise. 5. Raise the level of 'Die Keël' to encourage natural breaching. 6. Breach the estuary on a planned basis. The first three suggestions will probably result in the formation of a fresh-water lake, while the last three will maintain estuarine conditions.

1. *Discontinue artificial breaching*

The Bot River estuary has probably been breached naturally only twice in the past 70 years (Bally 1985). Left to its own devices it will form a fresh-water coastal lake

Table 1

Relative abundance of fish in the Bot River estuary. Abundance is given as total numbers captured (see Bennett *et al.* 1985 for details). Breeding area: E = estuaries, F = fresh water, S = sea. Habitat: R = estuarine resident, M = migratory, moving in and out of estuaries, MV = Marine, vagrant visitor to estuaries, LS = low salinities. Dependence on estuaries: AJ = adults & juveniles, J = juveniles, — = neither adult nor juvenile. Migration route: D = via mouth of Sonesta (when breached), K = via Kleinmond overflow. Mass mortality refers to numbers dying in low salinities during August 1981 (see Bennett 1985)

		ABUNDANCE							
		Seine net	Gill nets	Breeding area	Abundance cycle	Dependence	Migration route	Mass mortality	Lower lethal salinity
Gilchristella aestuarius	Estuarine round herring	14 913		E	R	AJ	—		<3
Psammogobius knysnaensis	Knysna sand goby	7 105		E	R	AJ	—		<3
Caffrogobius multifasciatus	Prison goby	7 071	45	E	R	AJ	—		<3
Clinus spatulatus	Bot river clinid	1 905		E	R	AJ	—		<3
Hepsetia breviceps	Cape silverside	64 490		E/S	R/M	—	D		<3
Syngnathus acus	Longnose pipefish	794		E/S	R/M	—	D	6	±3
Solea bleekeri	Blackhand sole		2	E/S	—	—			<3
Hyporhamphus capensis	Knysna halfbeak	8		E/S	—	J			<3
Lithognathus lithognathus	White steenbras	27	711	S	M	J	D	199	±3
Mugil cephalus	Flathead mullet	91	388	S	M	J	D/K		<3
Lichia amia	Leervis	6	204	S	M	J	D		<3
Rhabdosargus holubi	Cape stumpnose	5	21	S	M	J	D/K		<3
Monodactylus falciformis	Cape moony		17	S	M	J	D		<3
Liza richardsoni	Southern mullet	2 801	6 989	S	M	—	D/K	27	2–3
Galeichthys feliceps	Sea catfish		648	S	M	—	D	6 166	±3
Pomatomus saltatrix	Elf		367	S	M	—	D	3	±3
Rhabdosargus globiceps	White stumpnose		41	S	M	—	D	602	±3
Liza tricuspidens	Striped mullet		2	S	M	—	D		>5
Ophisurus serpens	Sand snake eel			S	M	—	D	117	±3
Argyrosomus hololepidotus	Kob			S	M	—	D	2	±3
Trigla capensis	Gurnard		28	S	MV	—	D		>5
Trachurus capensis	Maasbanker		26	S	MV	—	D		>5
Sarpa salpa	Strepie		12	S	MV	—	D		>5
Pomadasys olivaceum	Piggy		7	S	MV	—	D		>5
Diplodus cervinus	Zebra		4	S	MV	—	D		>5
Rhinobatus annulatus	Sandshark		1	S	MV	—	D		>5
Diplodus sargus	Blacktail		1	S	MV	—	D	8	>5
Engraulis capensis	Anchovy		1	S	MV	—	D		>5
Scomber japonicus	Mackerel		1	S	MV	—	D		>5
Cyprinus carpio	Carp	9	18	F	LS	—	—		—
Oreochromis mossambicus	Kurper	13	1	F	LS	—	—		—
Micropterus salmoides	Largemouth bass	—	2	F	LS	—	—		—

because river-water will progressively flush salts out of the system via the Rooisand overflow. The massive losses of water associated with breachings at Sonesta (together with the losses of plant life and invertebrates), will no longer occur. But salinities will drop, resulting in major ecological changes. *Ruppia* will probably be replaced with *Potamogeton*, a species which is more tolerant of low salinities and dominates many coastal lakes such as Swartvlei (Howard-Williams & Liptrot 1980). The two species are ecologically equivalent in many respects, so this change may not be of great consequence. Reeds such as *Scirpus* and *Phragmites* will spread, as they have done on Onrus estuary (Heinecken & Damstra 1983), and there will be an increasing likelihood of exotics such as *Salvinia*, *Myriophyllum* and *Eichornia* becoming established. Sediments will accumulate in the estuary because riverine deposits will no longer be flushed out as they are when the mouth is breached. The turbidity of the water will rise, because there will be no replacement of muddy waters with clean sea-water; and because the flocculation of suspended particles will decrease as the salinity drops (Willis 1985). Increased turbidity will reduce the area in which submerged weeds such as *Ruppia* and *Potamogeton* can grow. The invertebrate fauna will alter, with an increase in fresh-water insects and a decrease in estuarine and marine forms. The sand-prawn *Callianassa kraussi* will be unable to breed. Radical changes in the fish fauna can be anticipated, marine and estuarine species dying as salinities drop. Recruitment from the sea will be prevented for all marine species except, perhaps, the southern mullet, *Liza richardsoni*, which seems to be able to recruit via the Kleinmond overflow and channel (Bennett *et al.* 1985, Bennett 1985). Fresh-water species, particularly exotics such as the carp and bass, will take over. The herbivorous birds will benefit from the more stable water-levels and food supply; but the fish-eaters and more particularly the invertebrate-feeders, such as the flamingoes and the waders which feed on the exposed marginal sidements, will probably suffer and decrease in numbers.

Despite these substantial changes in the ecology, the choice of leaving the estuary unbreached is a viable option. It will, however, mean that the estuary will become a coastal fresh-water lake—in all likelihood it would already have become one if it had not been for human intervention. It will also mean that flooding of marginal properties will occur more frequently; but the more constant water-level may be more beneficial for yachtsmen. If nature conservation is the sole object to be achieved by a management plan, this option is an attractive one, for it allows the system to develop along natural lines.

2. *Construction of a weir across the mouth*

This suggestion was made to stabilize the water-level and reduce the normally massive loss of water that occurs after breaching the mouth at Sonesta. It has little to recommend it. A large and expensive structure would be needed, and it would still be necessary to breach the sand-bar behind the weir to allow water to flow out. There would be minimal tidal penetration and recruitment of fish would be hindered or prevented. Effectively, this option achieves no more than does the present natural overflow through Rooisand.

3. *Raise and fix the dunes*

This proposal was made to ensure that the Bot is never breached, and was coupled with a suggestion that a deeper channel be cut to link the estuary with Kleinmond. Again, this accomplishes little more than leaving the estuary untouched. Natural breachings are so rare that the expense of fixing the dunes would not be justified; and the canal would increase flooding of the Kleinmond estuary and the Lamloch swamps. We cannot recommend this option.

4. *Continued* ad hoc *breaching*

The historical reason for breaching the Bot at Sonesta was to allow entry of fish; but more recently the Cape Provincial Department of Nature and Environmental Conservation (which manages the estuary) has had repeated requests to breach the estuary because water-levels threaten adjacent properties, notably the yacht club which has a protective but ineffectual sea-wall. Indeed, in recent years the estuary has been breached increasingly frequently for this reason. The catastrophic effects on the ecology are documented above, and are reflected in the unusually low diversity of species (De Decker & Bally 1985), the enormous changes in the standing stocks in aquatic plant life (Bally *et al.* 1985), and the considerable reductions in bird-life that follow breaching (Heÿl & Currie 1985).

Ad hoc breachings that are based solely on water-level are to be avoided. But the motivation for breaching does highlight the need to position and protect properties on the margin of the lagoon in such a way that they are not threatened by high water-levels.

5. *Raising of 'Die Keël'*

In 1972 an attempt was made to raise the level of 'Die Keël'—a narrow shallow area between Rooisand and the Bot (Fig. 1). The object was to prevent the overflow of Kleinmond and thus increase the incidence with which the Bot River estuary breaches naturally. The attempt failed, waters from the Bot breaking through the elevated section of Die Keël, but much of it still remains elevated above the level of the rest of Rooisand. Blocking Die Keël would intensify the problem of marginal flooding in both the Bot and Kleinmond, since excess water would no longer be able to run off into the neighbouring estuary. It would also diminish the area shallow enough for growth of weeds in the Bot River estuary and intensify the loss of water and plants from the Bot should it breach naturally after reaching levels higher than normal.

This alternative cannot be recommended, since it will interfere with the Kleinmond estuary, interrupt the long-term natural development of connections between the Bot and Kleinmond estuaries, and heighten existing problems of flooding.

6. *Controlled breachings of the mouth*

If the system is to remain estuarine, periodic breaching is essential. It must be stressed that the motive is to maintain estuarine conditions, not to reduce water-levels. Estuarine conditions are primarily of benefit to the marine and estuarine fish and to fishermen. Breaching does, however, also periodically eliminate the water-weeds, which may be beneficial to yachtsmen; but is seems that the weeds are a problem only near the launching site, and a weed-cutter could be employed there (as it is at Sandvlei in False Bay).

If breaching is to be managed rationally, the position, timing and frequency of breaching are important. Sonesta is the obvious site, since the Rooisand site defeats the objective of maintaining estuarine conditions. The breaching should be undertaken when peak water-levels have been reached if a deep, long-lasting mouth is to be created, and if the maximum amount of sediment is to be scoured out. Peak water-levels usually occur in July to September (Fig. 2). However, early breachings will be detrimental to the recruitment of fish, for recruitment is highest in late spring to summer. To give an example, recruitment after the October 1981 breaching was substantially greater than that after the June 1983 opening (Bennett *et al.* 1985), and comparative data for the nearby Kleinmond and Palmiet estuaries reveal that recruitment is greatest between November and March (Bennett in prep.).

A final point influencing the timing of breaching is that greatest recreational use is made of the estuary during the summer holidays in December and January. If breaching occurs too late in the year, the estuary will not have time to refill, and the rotting weed will be left stranded at a time when most people will be using the estuary.

Taking all these factors into account, October seems the best month to breach the mouth, although it is recognized that this may be too late in the year for the estuary to refill before the holiday season.

Regarding the frequency of breaching, the estuary should remain closed as long as possible, allowing time for the system to stabilize, and the weed-beds to regrow. Heÿl & Currie (1985) estimate that it takes two to three years for the water-bird populations to build up again after a breaching. Prolonged closure of the mouth will also allow natural filling of the Kleinmond estuary and the Lamloch swamps via the overflow channels at Rooisand. It will also allow juvenile fish, which have been recruited while the mouth is open, to grow to a size attractive to anglers. But at the same time, these fish cannot breed in the estuary and must return to sea if they are to contribute to future fish stocks. To retain them in the closed estuary solely for anglers would be ecologically meaningless. Bennett *et al.* (1985) have analysed the growth rates of the dominant species and the ages at which they mature sexually. Elf mature when two years old, stumpnose and southern mullet at three, flathead mullet and leervis at four and white steenbras when five or more years old. Bennett *et al.* therefore suggest that the estuary be opened every three to four years to avoid a loss of reproductive potential by these fish.

As a practical measure, we suggest that the estuary be breached every three to four years *or* when salinities fall below 6‰ and threaten the most important marine and estuarine fish species. Under normal conditions it will take about four years for salinities to drop as low as this, although exceptional floods, (e.g. July 1983) can precipitately reduce salinities (Fig. 2).

If the Bot River estuary is to be breached at regular intervals opposite Sonesta it must be appreciated that there will be the following side-effects:

(a) The Bot River estuary and the Kleinmond swamps must be regarded as one

interconnecting system although they may be separated from each other in times of drought. The artificial breaching may therefore have a disrupting effect on the interconnections between the two major components of this ecosystem.

(b) Every time the mouth is opened artificially there is an input of marine sediment. The consequence is a tendency for the build-up of a dunefield at the footbridge between Sonesta and the existing dunes to the east of the mouth. The possibility may not be overlooked that this will eventually lead to the loss of the shallow-water area in front of the Sonesta Holiday Resort.

(c) Every time the mouth is opened artificially the entire ecological equilibrium of the Bot River estuary is seriously disrupted because of exposure and drying-out of the macrophyte beds of the vlei.

In effect, therefore, the breaching of the mouth will have an effect which is in direct contradiction to the natural processes governing this system.

CONCLUSION

No single management plan will satisfy all users of the Bot River estuary, but of those suggested only two are worth pursuing: either the estuary should never be breached (leaving it to develop into a fresh-water coastal lake) or it should be breached on a controlled basis in a manner determined by the ecological principles outlined above.

Whichever option is implemented, it is essential that the objectives for this decision be formulated first. We also believe that it is important that decisions are made after joint consultations between scientists, owners of land on the periphery of the estuary, users, and those authorities responsible for making and implementing decisions. The link between the Bot and Kleinmond estuaries also makes it important that people concerned with both systems be involved in any decisions on the future management of the Bot River estuary.

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