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THE FRESHWATER REQUIREMENTS OF ESTUARINE PLANTS INCORPORATING THE DEVELOPMENT OF AN ESTUARINE DECISION SUPPORT SYSTEM

Report to the
WATER RESEARCH COMMISSION
by the
DEPARTMENT OF BOTANY
UNIVERSITY OF PORT ELIZABETH

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THE FRESHWATER REQUIREMENTS OF ESTUARINE PLANTS INCORPORATING THE DEVELOPMENT OF AN ESTUARINE DECISION SUPPORT SYSTEM

VOLUME 1

by

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Report to the Water Research Commission on the project entitled

"The freshwater requirements of estuarine plants"

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EXECUTIVE SUMMARY

Much of the southern coast of Africa has a semi-arid climate by virtue of extended dry periods with high evaporation. The rainfall pattern is seasonal but not very reliable and the flow of freshwater into estuaries along this coast is especially low during the dry season and in dry years. Low runoff during dry weather is further diminished by the presence of dams in the catchment areas.

The severe drought of the 1980's highlighted the scarcity of our precious freshwater commodity. The Department of Water Affairs and Forestry implemented a programme of freshwater release from dams upstream of some estuaries to prevent hypersalinity, arising from natural evaporation. The scarcity of water resource distribution in South Africa and the need for maximum development of these resources focused attention on the research necessary to determine freshwater requirements of specific environments and especially estuaries. In 1989 the Water Research Commission provided funds to the Botany Department, University of Port Elizabeth to study the freshwater requirements of estuarine plants.

The first objective of the project was to collate all available information on the sensitivities of estuarine plants to freshwater related factors. The literature review (Volume 2) summarizes available information on phytoplankton, benthic microalgae and macrophytes. It also includes a synthesis of the existing floristic and abiotic data available from well studied South African estuaries.

South African estuaries are an important recreational asset and, because they cover only a small area of the coastline, their need for conservation is beyond question. The conservation of estuaries will depend on the ability to predict change in estuaries as well as an understanding of the consequences of change resulting from hypersalinity and water flow. The overall objective of the project was, therefore, to develop a decision support system that would aid in managing the freshwater supplies to estuaries. Such a decision support, or "expert system" should be able to predict plant response to freshwater-related physical factors such as salinity, water level and flow velocity. The development of the expert system met the second objective of the project i.e. "the development of a conceptual model of the multi-faceted role of freshwater in the maintenance of a viable estuarine plant ecology".

The first prototype expert system, EDSSys (Estuarine Decision Support System) was

developed by a postgraduate student in the Computer Science Department, University of Port Elizabeth, using the expert system shell, Personal Consultant Plus . The "knowledge" of EDSSys used was in the form of "if-then" rules and included the ecophysiological tolerances of estuarine macrophytes and the responses of phytoplankton to freshwater inflow. EDSSys was able to predict whether estuarine macrophytes would die, show reduced growth or be unaffected by a manipulation of freshwater inflow. It was also able to predict whether phytoplankton would bloom and form a feature of the estuary after the freshwater manipulation.

The Consortium for Estuarine Research and Management (CERM) was formed in 1993. Within the CERM group, co-ordination of existing estuarine models was conducted through their application to a case study, namely the Great Brak estuary. Different freshwater run-off scenarios were chosen on which to apply the model i.e. the natural run-off situation versus the Wolwedans dam full demand situation. Mike-11, a hydrodynamic model was used to produce salinity and water level data. With this information EDSSys was used to generate qualitative predictions of the estuarine floral response to different freshwater run-off scenarios.

The second prototype expert system, PEDSSys (Plant estuarine decision support system) was developed by Dr M. Brinck of MarBri Strategies. PEDSSys was developed using the expert system shell dmX in a Windows environment. PEDSSys uses bilinear logic instead of "if-then" rules. The results in PEDSSys for the estuarine macrophytes are based on growth rate adjustment with a score range of -10 to +10. The score for phytoplankton and benthic microalgae is based on biomass and the score range is 0 to 10. Seasonality, with regard to plant responses, was included in PEDSSys. For example the intertidal macrophyte *Sarcocornia perennis* is more sensitive to prolonged inundation during the growing season, i.e. spring/summer.

Knowledge for the expert system, i.e. salinity and inundation tolerances, for specific macrophyte species, were obtained from the literature. Ecophysiological data is plentiful for overseas species but was found to be lacking for typical South African species. Estuarine macrophyte studies in South Africa have mainly concentrated on descriptive ecological studies. A few studies have documented ecophysiological tolerances (Breen *et al.* 1977, Naidoo and Rughunanan 1990, Naidoo and Naiker 1992a, Naidoo and Naidoo 1992b) of mainly Natal species i.e. *Sarcocornia natalensis* but not those species common to east and south coast estuaries. Because of the paucity of ecophysiological response data, laboratory studies were conducted on selected estuarine macrophytes.

The third objective of the project was to use laboratory and field measurements to quantify the major aspects of the conceptual model. The macrophytes chosen to study were:

Submerged macrophytes: *Ruppia cirrhosa* and *Zostera capensis*
Salt marsh plants: *Sarcocornia perennis* and *Spartina maritima*
Emergent macrophytes: *Phragmites australis*.
These species were chosen because of their abundance in Cape estuaries.

Salinity and water level fluctuations were identified as important freshwater-related physical factors which affect macrophyte growth. The effect of salinity and inundation on these plants was studied and the results used as rules for the PEDSSys expert system. The results of field observations and laboratory experiments are reported.

Submerged macrophytes:

Laboratory studies showed that *Zostera capensis* grew best between 15 and 35 ppt *Ruppia cirrhosa* grew best in freshwater but survived in 75 ppt. *Ruppia cirrhosa* can tolerate fluctuating salinity and it was found to recover after exposure to high salinity. *Ruppia cirrhosa* is an opportunistic species adapted to its habitat in periodically closed estuaries. These systems are characterized by fluctuating salinity because of the erratic freshwater input. Although *R. cirrhosa* will grow vegetatively in hypersaline water (> 40 ppt) this study showed that no seeds germinated where the salinity was greater than 35 ppt.

Despite its wide salinity tolerance range it is apparent that *R. cirrhosa* is absent from marine intertidal habitats where *Z. capensis* is common. This appears to be related to differences in desiccation tolerance. *Zostera capensis* plants exposed for 2h took one day to recover, whereas *R. cirrhosa* plants took four days. Daily exposure of 5h caused complete die-back of *R. cirrhosa* within five weeks, but *Z. capensis* survived these repeated drying cycles. *Zostera capensis* has a leaf sheath that protects the basal meristem and new leaves grow rapidly from this area within a few days. Neither plant recovered from an exposure of one week. In the field, the rate of desiccation will depend on ambient conditions and sediment water-holding capacity. Although *R. cirrhosa* cannot survive in intertidal habitats, it is adapted to ephemeral habitats that dry out periodically. *In situ* these plants grow rapidly from a large seed bank, once water levels rise.

Salt marsh macrophytes:

Sarcocornia perennis is a succulent that occurs in the lower intertidal zone of salt marshes. This plant grows best in a salinity environment less than 35 ppt, and in saturated substrates. The highest mortality occurs in the treatment where plants are

completely submerged. Submerged plants decomposed rapidly. This is especially in the case of low salinity environments (< 15 ppt).

Spartina maritima (rice grass) occurs lower in the intertidal zone than does *S. perennis* and grows equally well whether submitted to tidal or completely submerged conditions. *Spartina maritima* can survive submergence for longer periods than other plants and therefore occupies the lower elevation of salt marshes. Growth is reduced in dry treatments and where salinity levels are greater than 35 ppt. *Spartina maritima* is not found in periodically closed South African estuaries. This is likely due to the plant's requirement for tidal flooding and saturated substrates.

Emergent macrophytes:

Field surveys showed that common reed, *Phragmites australis* which is normally associated with freshwater, occurs at sites of freshwater seepage in marine dominated estuaries. Plant height increased away from the water's edge in these estuaries and measurements showed that this could be related to a decrease in substrate salinity. Laboratory studies demonstrated that *Phragmites* can survive tidal inundation with saline water (35 ppt) if the roots and rhizomes are located in freshwater. Plants whose roots were supplied with 20 ppt salinity showed signs of stress after two weeks.

Microalgae:

Phytoplankton dominance in estuaries has been associated with increases in river flow both locally (Haw 1984, Allanson and Read 1987 and Hilmer 1990) and overseas (Flint 1985, Malone *et al.* 1988). Studies in eastern Cape estuaries (Hilmer 1984, Hilmer 1990, Hilmer and Bate 1990, 1991) found that freshwater input supported phytoplankton communities as freshwater brought in nutrients and maintained stable stratified conditions. These latter studies focused on one region only, and therefore, it was necessary to test and quantify the data for other Cape estuaries. This study investigated the influence of freshwater on phytoplankton in a number of other Cape estuaries.

The relationship between freshwater input and phytoplankton response is that high biomass (20 μg chlorophyll-a l^{-1}) can only be maintained if the nitrate concentration of the freshwater is greater than 200 μg l^{-1}. Water residence time must be adequate to create stable stratified conditions to allow phytoplankton to bloom. Freshwater pulses of less than 200 μg l^{-1} nitrate into the Keurbooms and Great Brak estuaries increased nutrient levels and raised chlorophyll-a concentrations but to less than 20 μg chlorophyll-a l^{-1}.

In 1992 studies on benthic microalgae commenced. The hypothesis tested was that benthic microalgal productivity increases in estuaries where a reduction in freshwater flow has limited productivity in the water column by excluding freshwater supplies of nutrients. Before this could be tested, suitable methods for measuring benthic microalgal chlorophyll-*a* had to be investigated. The measurement of estuarine benthic chlorophyll-*a* is complicated by the presence of chlorophyll-*a* degradation products as well as other pigments. It was found that cores of 20 mm internal diameter extracted with 30 ml of ethanol, yielded the highest chlorophyll-*a* per unit of substrate extracted. The highest chlorophyll-*a* concentrations were obtained for the top 10 mm of sediment. This was taken as a reliable sampling depth for estimating benthic microalgal chlorophyll-*a*.

A comparison between spectrophotometer and HPLC chlorophyll-*a* values showed that the spectrophotometer overestimated chlorophyll-*a* by a mean value of 20 %. The traditional spectrophotometric technique for measuring chlorophyll-*a* is not a reliable method in sediments because pigments other than chlorophyll-*a* interfere with the spectrophotometric reading. The most satisfactory technique for measuring chlorophyll-*a* in sediments is the *High Pressure Liquid Chromatography (HPLC) method*. Chromatographic techniques such as liquid chromatography physically separate the different photosynthetic pigments before spectrophotometric readings are taken.

This raises problems where samples are taken in areas where HPLC instrumentation is not available. The degradation rate of microbenthic chlorophyll-*a* under refrigerated conditions was, therefore, studied over a period of one week. The degradation rate was linear over a period of 7 days and the value of the degradation slope was 10.63. By multiplying 10.63 by the number of days since sampling, and then adding this factor to the actual measured chlorophyll-*a*, the correct original chlorophyll-*a* value can be obtained. Using this correction factor, samples taken a long distance away from the laboratory can be brought back and subsequently analyzed.

Detailed studies in the Swartkops, a permanently open estuary, showed that low chlorophyll-*a* was found in the mouth area. This was related to sediment resuspension. The sediment surface in the middle reaches of the estuary provided favourable growth conditions for benthic microalgae. This could be attributed to the higher silt content in the sediments. Species composition analysis showed that diatoms were the dominant group of the microbenthic algal community.

Estuaries were sampled along the Cape south and south-east coast during August 1992 to compare phytoplankton and benthic microalgal biomass. Higher chlorophyll-*a* concentrations were observed in the sediment than in the water column for all systems except the Sundays and Gamtoos estuaries. These data show that the benthic

component would play an important role in energy fixation and the food web of these estuaries.

The identification of microalgae is proceeding. These identification studies are necessary in order to determine whether species or groups are related to the salinity status of estuaries and whether the benthic species are specific to the benthos or detritus residues of planktonic origin.

Macrophyte distribution studies:

In 1990 the distribution of estuarine macrophytes in relation to freshwater was studied in a number of eastern Cape estuaries (Seekoei, Kabeljous, Kromme and Gamtoos). A continual flow of freshwater into estuaries was shown to create a salinity gradient along which different macrophyte communities were distributed. In systems with no freshwater inflow there was no spatial separation of macrophytes. Salt marshes and the submerged macrophyte *Zostera capensis* which are normally found at the marine end of estuaries extended into the upper reaches where freshwater inflow is stopped. In small estuaries, salinities fluctuated because of the major influence of periodic small floods. When estuaries are in a stable, brackish condition (13 - 28 ppt), they are dominated by dense *Ruppia cirrhosa* communities which are normally associated with lower salinity regions.

Overall, the macrophytes can be separated into supratidal, intertidal and submerged communities. Within each of these, salinity determines the distribution of the species and, in the case of the submerged macrophytes, influenced species composition. The salinity of supratidal salt marsh sediments is not dependent on the adjacent estuarine water body. Hence, while freshwater input into estuaries is necessary to maintain the integrity of submerged and emergent communities it is possibly not a requirement for supratidal marshes. The salinities of the intertidal sediments are controlled by the estuarine water. High water column salinities (35 ppt) result in the accumulation of salts in the intertidal zone which then reduce the distribution of macrophytes.

Most of the known South African emergent macrophytes were found in all of the four estuaries studied. Species diversity and the extent of emergent communities appear to be related to topography and available physical space. Species diversity was therefore higher in the larger Gamtoos and Kromme estuaries. The low diversity and limited emergent macrophyte cover in the Seekoei estuary is most likely due to fluctuating water levels and periodic hypersaline conditions.

The large salt marsh areas in the Gamtoos and Kromme estuaries are dependent on an

open mouth and daily tidal exchange. If the mouths were to close and tidal flushing stop the marshes would become hypersaline and productivity would decrease. Flushing of the marshes would be reduced, restricting nutrient exports to the estuary and nearshore environment. Under high rainfall conditions, sediment salinities would be decreased, creating favourable conditions for encroachment by terrestrial species. There is a need to conserve the salt marshes in the eastern Cape. O'Callaghan (1990) has shown how poorly developed the halophytic vegetation is along the False Bay coast due to human impacts and reduced saline inputs.

The fourth objective of the project was to make recommendations regarding the management of freshwater discharge to estuaries. These recommendations are summarized as:

1) The freshwater supplies to estuaries should be protected to the extent that these systems, which are highly productive, are not lost to either land or marine-dependent environments.

2) In permanently open estuaries, freshwater should be discharged into estuaries in such a manner as to maintain an open mouth, so as to achieve the tidal flushing essential for salt marsh environments.

3) Where the normal flow of freshwater is reduced, aquifer water should be strongly protected in order to maintain nodes of freshwater/brack dependent species.

4) To maintain evenly distributed supplies of estuarine biota for recruitment, no plans should be drawn up which would totally destroy any estuary.

5) Where it is necessary to reduce freshwater flow, periodic freshwater flushing which would simulate a natural flood should be managed at intervals (possibly 1 year in 5).

6) South African estuaries must be classified into types based on vegetation abundance and distribution. On the basis of the classification protected systems should be proclaimed at intervals along the coast so as to provide an even distribution of types which can be managed around the whole coastline. We further recommend that they are managed by the National Parks Board.

This project has resulted in the development of a decision support system that is available for use by water managers for the prediction of estuarine plant responses to different freshwater run-off scenarios. As additional knowledge becomes available and comments

are received from people using the expert system it will need to be revised and updated. It is strongly recommended that once the expert system is found to be useful, additional plants are added to the system. Studies should be expanded to include Natal estuaries and species.

Any study on the sensitivity of estuarine plants to salinity can be projected endlessly. This project has identified that estuarine plants are sensitive to both salinity and water level changes and that such sensitivity is caused by limiting normal freshwater catchment discharges. The project was extended into an examination of microbenthic algae when data became available to demonstrate that the amount of chlorophyll-a in the benthic component exceeded that in the water of most south Cape systems by orders of magnitude. It has not been possible to complete this extended section with respect to salinity sensitivity. It will not be possible to achieve such objectives before the taxonomy of the species is complete and we know which organisms to work with. We recommend that studies on phytoplankton and microbenthic species be continued at a steady pace but on a low-cost basis.

It is important that spatial vegetation models are developed so that dynamic processes can be mapped. Work has already begun in this regard. In collaboration with Prof J. Hearne (Department of Maths and Applied Maths, University of Natal, Pietermaritzburg) a dynamic vegetation model is being developed under the auspices of CERM. The development of GIS (Geographical Information Systems) spatial models are important for estuarine management as plant community changes in response to physical conditions can then be mapped and spatially displayed. An example of this is the spread of emergent macrophytes in response to increased sedimentation and decreased salinity.

It is recommended that basic research is focused on:

a) the importance of freshwater seepage from tributaries leading into estuaries.
b) understanding the interaction of the salt marsh with the physical environment.
c) co-ordination of hydrodynamic processes and phytoplankton response.
d) contribution of benthic microalgae to primary production in South African estuaries.
e) microfloral species identification.

Finally it is recommended that the floral and faunal response to freshwater-controlled physical factors be linked so that a community response to freshwater input can be identified.

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The Steering Committee responsible for this project, consisted of the following persons:

Dr PCM Reid	:	Water Research Commission (Chairman)
Mr D Huyser	:	Water Research Commission (Secretary)
Mr HC Chapman	:	Water Research Commission
Prof CM Breen	:	University of Natal
Prof OAM Lewis	:	University of Cape Town
Prof TH Wooldridge	:	University of Port Elizabeth
Dr MMB Talbot	:	Talbot and Talbot
Dr AK Whitfield	:	JLB Smith Institute
Dr PJ Ashton	:	WATERTEK/CSIR
Mr P Huizinga	:	EMATEK/CSIR
Mr PD Morant	:	EMATEK/CSIR
Mr CA Bruwer	:	Department of Water Affairs and Forestry

All members of the Steering Committee are sincerely thanked for their valuable input, not only at the annual committee meetings but throughout the duration of this project. Ms J. Slinger and Mr L. Barwell of EMATEK are also thanked for their input.

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LIST OF ABBREVIATIONS

A:	Core area (in mm^2)
ANOVA:	Analysis of variance
CERM:	Consortium for Estuarine Research and Management
Chl-*a*:	Chlorophyll-*a*
DCCA:	Detrended Canonical Correspondence Analysis
df:	degrees of freedom
E_{b665}:	Absorbance before acidification at 665 nm
E_{a665}:	Absorbance after acidification at 665 nm
EDSSys:	Estuarine Decision Support system
FRD:	Foundation for Research and Development
Fm:	Maximum fluorescence
Fo:	Intial fluorescence
Fv:	Variable fluorescence
H':	Shannon-Weaver diversity index
GIS:	Geographical information systems
PEA:	Plant Efficiency Analyzer
PEDSSys:	Plant Estuarine Decision Support system
HPLC:	High performance/pressure liquid chromatography
ppt:	parts per thousand
PS II:	Photosystem II
SD:	Standard deviation
SE:	Standard error
SEM:	Scanning electron microscope
spec:	Spectrophotometer
V:	Volume of solvent

GLOSSARY OF TERMS

ACCLIMATION
the process by which a range of adjustments occur within the plant or system.

ALLOCHTHONOUS
not made *in situ* (i.e. not made in the estuary but imported from outside the syster

BRACKISH
water within the salinity range of 5 - 15 ppt (seawater = 35 ppt)

ECOTYPE
the product arising as a result of the response of the organism to the particular habita which it lives. *e.g. Plantago maritima* has a height of about 17,5 cm in waterlogged mud and 56 m in a fertile meadow.

EPIPHYTE
a plant that lives on the surface of other plants but does not derive water or nourishment from them.

EUPHOTIC ZONE
zone (either water or sediment) into which sufficient light penetrates for active photosynthesis.

EUTROPHICATION
a process by which pollutants cause a body of water to become over-rich in organic and mineral nutrients, so that algae grow rapidly and deplete the oxygen supply.

Fv/Fm
The ratio of variable fluorescence to maximal fluorescence. When light is first made available to plants, fluorescence (re-emission of light) reaches its initial level (Fo) and after about 0.15 s it rises to a maximum value, Fm. Variable fluorescence Fv is equal to Fm-Fo.

GLYCOPHYTE
a plant unable to tolerate saline conditions.

HALOPHYTE
a plant that is tolerant of high concentrations of salinity.

HYPERSALINE
salinity greater than 40 ppt

IN SITU
in the natural habitat or within the organism

PHENOLOGY
the study of periodicity phenomena of plants, e.g. time of flowering in relation to climate.

PHYTOPLANKTON BLOOM
dense growth of algae or phytoplankton.

PRIMARY PRODUCTION
fixation of inorganic carbon etc. into organic matter by autotrophs, which are referred to as primary producers.

RETENTION TIME
this is an estimate of the average length of time that a water/solute concentration remains at a particular level in an estuary. Other terms commonly used are flushing time or mean detention time.

SALINITY
the proportion of salts in pure water, in parts per thousand by mass. Units expressed as ppt, g l^{-1}, g kg^{-1}. In this report ppt was used.

1. INTRODUCTION

The severe drought of the 1980's highlighted the scarcity of our precious freshwater commodity. This focused research on the freshwater requirements of specific environments and especially estuaries. In 1989 the Water Research Commission provided funds to the Botany Department, University of Port Elizabeth to study the freshwater requirements of estuarine plants. The overall aim of the study was to establish a basic understanding of the manner in which freshwater controls the plant ecology of estuaries. This information was then to be used to provide a predictive capability of estuarine response to varying freshwater regimes. Further it would be used to formulate sound policy guidelines for the management of estuarine ecosystems in the context of limiting freshwater resources.

Broadly, the objectives of the project were:

a) the collation of available information on responses to freshwater of estuarine macrophytes and phytoplankton,

b) the development of a conceptual model of the multi-faceted role of freshwater in the maintenance of a viable estuarine plant ecology,

c) laboratory and field measurements to quantify the major aspects of the conceptual model, and

d) to make recommendations regarding the management of freshwater discharge to estuaries.

The conservation of estuaries is dependent on being able to predict change and on understanding the consequences of change. A decision support system was, therefore developed to aid in the management of the freshwater requirements, with special reference to plants. Such a decision support or "expert system" should predict plant responses to freshwater-related physical factors such as salinity, water level and flow velocity. The first prototype expert system, EDSSys was developed using the expert system shell, Personal Consultant Plus. The knowledge EDSSys used was in the form of "if-then" rules and included the ecophysiological tolerances of estuarine macrophytes and

the response of phytoplankton to freshwater inflow. EDSSys predicted whether macrophytes would die, show reduced growth or be unaffected by the manipulation of freshwater inflow. It also predicted whether phytoplankton would bloom and form a feature of the estuary after the manipulation.

The second prototype expert system, PEDSSys (Plant estuarine decision support system) was developed by Dr M. Brinck of MarBri Strategies. PEDSSYs was developed using the expert system shell dmX in a Windows environment. PEDSSys uses bilinear logic instead of "if-then" rules. Results for estuarine macrophytes are based on growth rate adjustment with a score range of -10 to +10. The score for phytoplankton and benthic microalgae is based on biomass and the score range is between 0 and 10.

Knowledge for the decision support systems i.e. salinity and inundation tolerances for specific macrophyte species, was obtained from the literature and from controlled laboratory studies on selected estuarine macrophytes. The macrophytes chosen to study were:

Submerged macrophytes:	*Ruppia cirrhosa* and *Zostera capensis*
Salt marsh plants:	*Sarcocornia perennis* and *Spartina maritima*
Emergent macrophyte:	*Phragmites australis*.

These specific species were chosen because of their abundance in Cape estuaries.

Salinity and water level fluctuations were identified as important freshwater-related physical factors which effect macrophyte growth. The effect of salinity and inundation on these plants was studied and the results were used as "knowledge/rules" for the expert system. Plant tolerance was studied in the laboratory over a wide salinity range. The reason for this was that in small temporarily closed estuaries and in estuaries where there is reduced freshwater input, high salinity levels (> 45 ppt) have been observed to cause impoverishment of the estuarine flora (Adams *et al*. 1992). In addition to salinity stress, estuarine plants are subjected to stress from waterlogging and prolonged inundation. For example, dam construction in the catchment of the Great Brak estuary (34°03'S, 22°14'E) has increased the frequency and duration of mouth closure. During closed mouth conditions, water levels rise due to localized freshwater run-off. This causes salt marshes to be inundated for extended periods. For this reason, management decisions are required regarding the length of time intertidal plants can survive under prolonged submerged conditions before freshwater needs to be artificially released from the dam

and the mouth of the estuary mechanically opened.

Salt marshes are important estuarine plants communites as they serve as zones of nutrient production and retention and as feeding and shelter areas for both marine and estuarine organisms.

The distribution of estuarine macrophytes in relation to freshwater input was studied in the Gamtoos, Kabeljous, Seekoei and Kromme estuaries. A number of sampling stations were selected along the length of each estuary and floristic and environmental variables (salinity, nutrient concentrations and turbidity) were measured in quadrats placed at intervals across the width of the estuary. The multivariate analysis techniques of ordination and classification were used to determine the relationship between macrophyte distribution and environmental variables within estuaries and between estuaries.

Phytoplankton dominance in estuaries has been associated with increases in river flow both locally (Haw 1984, Allanson and Read 1987 and Hilmer 1990) and overseas (Flint 1985, Malone *et al*. 1988). Studies in eastern Cape estuaries (Hilmer 1984, Hilmer 1990, Hilmer and Bate 1990, 1991) found that freshwater input supported phytoplankton communities as freshwater brought in nutrients and maintained stable stratified conditions. Because these studies focused on only one region it was necessary to test and quantify the data for other Cape estuaries. The study was, therefore, extended to investigate the influence of freshwater on phytoplankton in a number of other Cape estuaries, and especially estuaries along the south coast where water is not in short supply.

In 1992, studies on benthic microalgae were initiated in order to establish whether benthic primary productivity was increased in estuaries where reduced freshwater input limited the productivity of the water column. However, before this could be established, methods for measuring benthic chlorophyll-a had to be investigated. Appropriate methods for chlorophyll-a measurement of benthic microalgae were investigated in detail in the Swartkops estuary. Estuaries were sampled along the Cape south and south-east coast during August 1992 to compare phytoplankton and benthic microalgal biomass. The identification of phytoplankton and benthic microalgae to species level, using the scanning electron microscope began in 1993.

2. THE DISTRIBUTION OF ESTUARINE MACROPHYTES IN RELATION TO FRESHWATER

Introduction

The objective of this study was to compare the distribution of macrophytes in four local estuaries and relate these to controlling environmental variables. Freshwater is a single environmental variable, but, because its rate of inflow is difficult to quantify, associated variables such as salinity and nutrient concentrations were used as an index of freshwater input. Well known statistical treatments were employed to determine whether any relationship existed between macrophyte distribution and environmental variables between estuaries, and also to assess whether these estuaries could be separated according to different macrophyte assemblages as well as on the basis of freshwater input.

The four estuaries studied were the Gamtoos, Kabeljous, Seekoei and Kromme. They were chosen because they are geographically very close to each other yet they have very different freshwater inputs. The Gamtoos has a large catchment and has a continuous freshwater input which creates a salinity gradient up the whole length of the estuary. In contrast, construction of the Churchill and Charlie Malan dams in the catchment of the Kromme has restricted freshwater input into this latter estuary. The Seekoei and Kabeljous are ephemeral estuaries. They have small catchments causing freshwater input to be erratic as they are dependent on the highly variable and seasonal rainfall.

Methods

Study sites

Sampling was carried out in four estuaries in St. Francis Bay on the south eastern Cape coast (Fig. 1). The distance between the two estuaries which are furthest apart is 45 km and all experience the same climate. However the estuaries differ considerably in morphology and freshwater inputs. General estuary characteristics are summarized in Table 1.

The Gamtoos estuary has the largest catchment and therefore the greatest mean annual runoff (Table 1). The mouth is permanently open and it has a narrow intertidal area as the channel is deeply eroded into the wide river floodplain (Reddering and Esterhuysen 1984a). The Gamtoos estuary has three large dams situated on its tributaries: the Paul Sauer dam on the Kouga river (capacity 132 x 10^6 m^3), Beervlei dam on the Groot river tributary (capacity 93.5 x 10^6 m^3) and the Loerie dam (capacity 3.92 x 10^9 m^3). The sum of the capacities of these dams is equivalent to half the mean annual run-off (Heinecken 1981) but although freshwater flow is reduced there is a continuous input into the system.

The Kabeljous and Seekoei are small estuaries with small catchments and low mean annual runoffs (Table 1). Their mouths are blocked by sandbars which are breached naturally only during floods. These estuaries thus have the characteristics of lagoons with occasional estuarine phases. They are therefore referred to as 'blind estuaries' but the term 'ephemeral estuary' is preferred (Reddering and Esterhuysen 1984b). Whitfield (1992) classified these systems as temporarily open/closed estuaries. Obstructions in the catchments of these estuaries consist of numerous small farm dams situated on the tributaries. Such dams have been shown to have a big effect in catchments with low run-offs, particularly during dry years (Bickerton and Pierce 1988). In 1973 a causeway was constructed 700 m upstream of the Seekoei mouth. This causeway blocks tidal flow, reduces the scouring effect of floods and causes marine sand to accumulate between the causeway and the sea which cuts the estuary off from the sea (Bickerton and Pierce 1988).

The mouth of the Kromme estuary is permanently open. The catchment is larger than either the Kabeljous or Seekoei estuaries but it has two major dams. The Churchill dam was constructed in 1943 some 36 km above the tidal reach of the estuary and has a capacity of 33.3 x 10^6 m^3. In 1982 the Charlie Malan dam was constructed below the Churchill dam, only 4 km above the tidal reach of the estuary. It has a capacity of 100 x 10^6 m^3 but has only been filled twice since construction. If the capacities of these dams (133 x 10^6 m^3) are compared with the mean annual run-off (123 x 10^6 m^3) of the Kromme catchment (Table 1), it is apparent that on average very little water discharges into the estuary. The dams dampen the effect of all floods smaller than the 1-in-30 year flood (Bickerton and Pierce 1988, Fromme and Badenhorst 1987). A uniform salinity of 35 ppt exists up the length of the estuary which has, as a result, become a side-arm of the sea.

Table 1 Summary of estuary characteristics (Day 1981a and Jezewski and Roberts 1986).

	Gamtoos	Kabeljous	Seekoei	Kromme
ESTUARY LOCATION	33°58'S 25°04'S	34°00'S 24°56'S	34°05'S 24°55'S	34°05'S 24°51'S
ESTUARINE AREA (ha)	175	82	98	275
CATCHMENT AREA (km^2)	34450	262	250	1085
MEAN ANNUAL RUNOFF (10^6 m^3)	500.6	16.3	15.7	123
ESTUARY LENGTH (km)	20	2.25	3.5	14

Figure 1 Map indicating the location of the Gamtoos, Kabeljous, Seekoei and Kromme estuaries.

Sampling strategy and measurement of environmental variables

In March 1990 the distribution of macrophytes in the four estuaries was determined by selecting a number of sampling stations along the lengths of the four estuaries. At each station quadrats (0.25 m^2) were sampled at intervals across the width of the estuary.

For each quadrat the cover for each species was estimated visually. These data were converted to the Braun Blanquet cover abundance scale (Mueller-Dombois and Ellenberg 1974) for use in multivariate analysis. Sediment cores were collected from each quadrat and split into top (0 - 30 mm) and bottom (30 - 70 mm) layers. Environmental variables recorded from each quadrat included water content, organic matter content, salinity and nutrients (nitrate nitrogen, available phosphorus and total phosphorus) of the sediments. Each quadrat was assigned to an elevation class: 1 permanently submerged (> 0.5 m water depth); 2 periodically submerged (< 0.5 m water depth); 3 lower intertidal; 4 middle intertidal; 5 upper intertidal and 6 supratidal.

Sediment water content was determined as a percentage of the dry weight and the organic matter content was determined after combustion at 600°C for 5 h. For the determination of sediment salinity, an air dry sample of approximately 5 g was mechanically shaken with 25 ml distilled water for 20 minutes. The suspension was filtered through Whatman No. 1 filter paper and the supernatant was made up to 25 ml. Salinity was determined by the Mohr titration as described by Strickland and Parsons (1972).

The copper cadmium method for the determination of nitrate-nitrogen was used (Bate and Heelas 1975). The available sediment phophorus was measured using a combination of methods by Olsen and Dean (1965) and Strickland and Parsons (1972). A mixed-acid digestion procedure (Olsen and Dean 1965) was used to determine the total phosphorus content in the sediment.

Analysis of multispecies distribution patterns

The multivariate techniques of ordination and classification were used to detect pattern/structure in the macrophyte communities of the estuaries. Classification was used to determine whether estuaries could be classified according to different macrophyte assemblages. Use was made of the two-way indicator species analysis (TWINSPAN, Hill 1979). TWINSPAN produces a classification of samples by the progressive splitting of ordinations (reciprocal averaging) at their centers of gravity. At each split of the dichotomy, indicator (diagnostic) species are chosen to define the two groups of data.

With the aid of the computer program CANOCO (ter Braak 1985), Detrended Canonical Correspondence Analysis (DCCA) was used to determine important environmental variables which might be controlling macrophyte distribution patterns between estuaries. DCCA is a constrained ordination technique that produces simultaneous results based on species abundance and environmental variables (ter Braak 1986). Species-environment correlations were used to interpret the ordination axes. By examining the signs and magnitudes of these coefficients one could infer the importance of each variable for prediction of species composition (ter Braak 1986).

Diversity index

The Shannon-Weaver diversity index (H') was calculated for the different estuaries. Species diversity indices are the ratios between the number of species and importance values (numbers, biomass, production) (Odum 1971).

Results

Macrophyte distribution

Gamtoos river estuary

Relatively few submerged macrophytes were found in the Gamtoos estuary and, although dense *Potamogeton pectinatus* L. beds did occur above the tidal reach of the estuary, they are not directly part of the estuarine system and were excluded from this study. Knoop (pers. comm.) documented in 1989 the presence of the alga, *Caulerpa filiformis* (Suhr) Hering towards the west of the mouth region and leading into the blind arm.

Emergent macrophytes in the Gamtoos were concentrated in the upper and lower estuarine reaches (Fig. 2). The middle reaches of the Gamtoos estuary were devoid of vegetation except for isolated monospecific outcrops of *Phragmites australis* (Cav.) Trin (Fig. 2). The upper mesohaline reaches (5 - 18 ppt) of the estuary were dominated by a dense brackish reed community of *Phragmites australis* and *Scirpus maritimus* L. In the lower estuarine reaches the broad flat area around the blind arm (pre-1971 channel) has provided an ideal region for halophyte colonization and salt marsh formation.

Kabeljous river estuary

Submerged macrophytes covered 31.62 ha which was the largest in the estuary by comparison with emergent macrophytes which covered only 8.08 ha. *Ruppia cirrhosa* (Petagna) Grande was the dominant submerged macrophyte and occurred rooted in the substrate in contrast to the filaments of free-floating *Cladophora sp.* which were found intermingled with it. Scattered salt marsh species were found on the floodplain bordering the estuary (Fig. 3). Dense stands of *Phragmites australis* occurred above the old National road. Water movement in this area has been restricted by the construction of the road and a railway bridge. The resultant shallow water conditions have favoured colonization by *Phragmites*. This supports the findings of Weisser and Howard-Williams (1982) and Roman *et al.* (1984) who have shown that a restriction in tidal flow and associated shallow freshwater conditions encourage *Phragmites* growth.

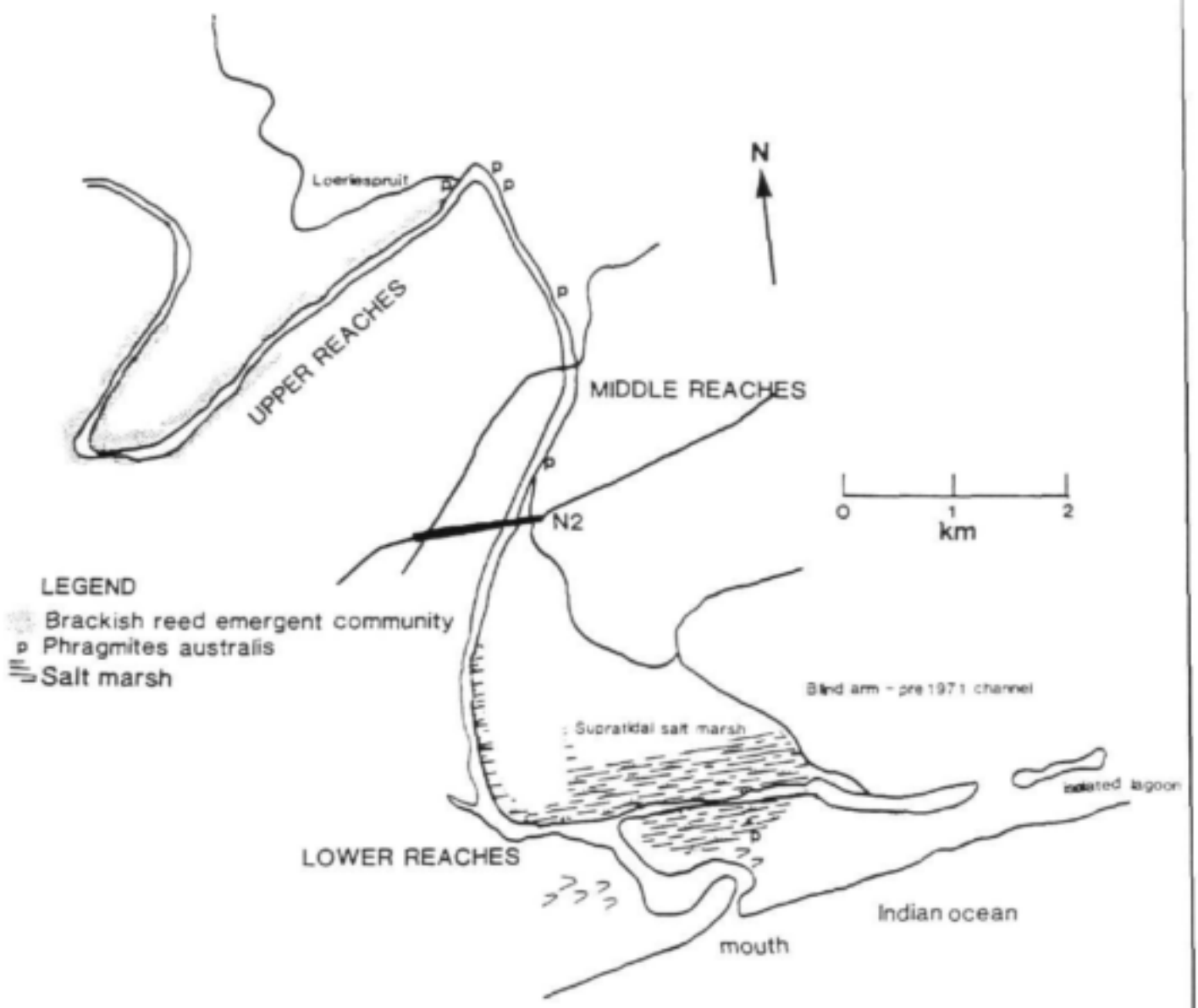


Figure 2 The distribution of macrophytes up the length of the Gamtoos estuary.

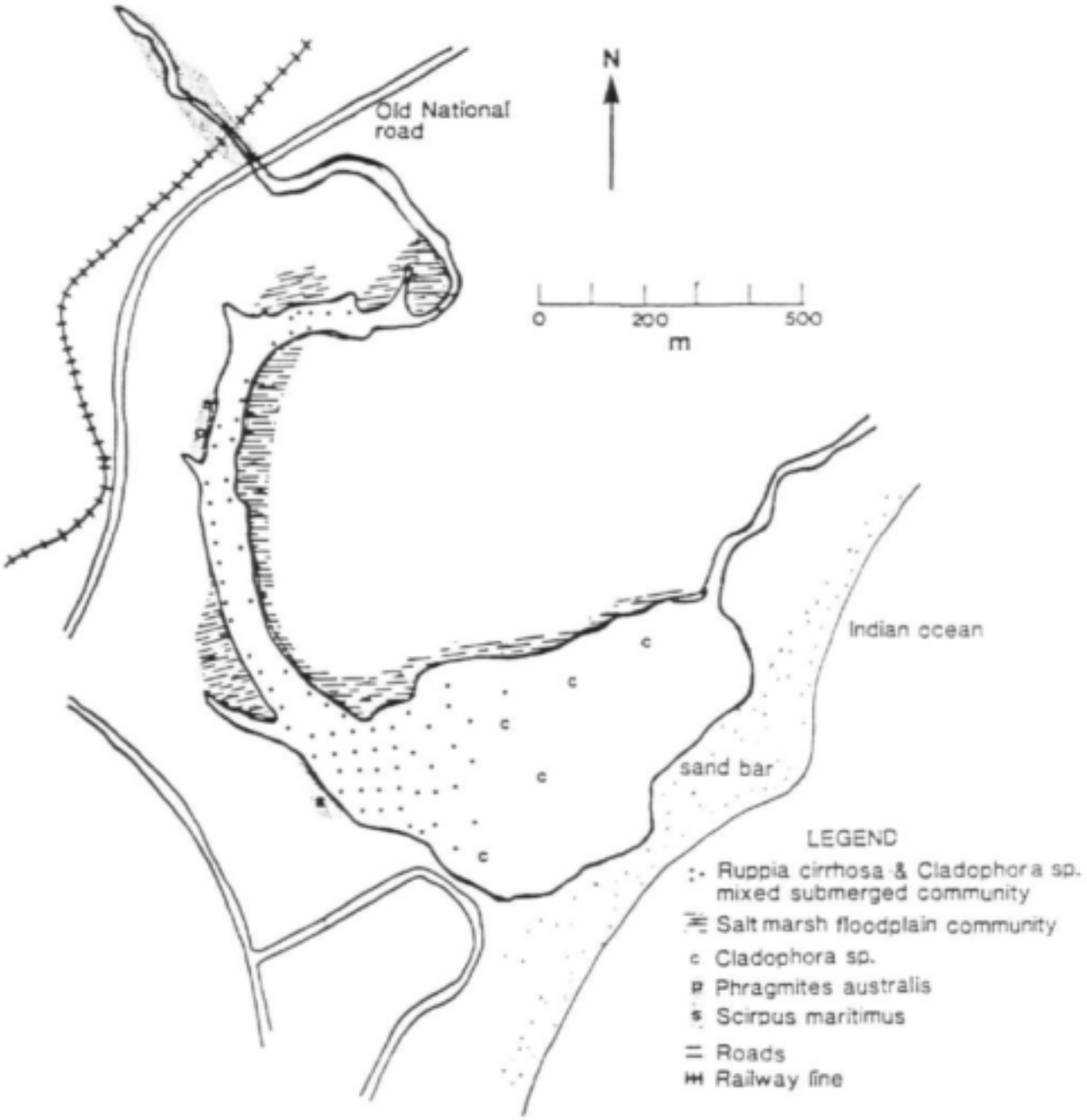


Figure 3 The distribution of macrophytes up the length of the Kabeljous estuary.

Seekoei river estuary

Submerged macrophytes covered 29.4 ha while emergent macrophytes covered only 2.98 ha (Fig. 4). Dense beds of *Ruppia cirrhosa* were found in the narrow channels of both the Seekoei estuary and the Swart river which is a side-arm of the Seekoei (Fig. 4). Macroalgae found included *Cladophora sp.*, *Enteromorpha sp.* and *Ectocarpus sp.*

The floodplain towards the west of the causeway was covered by scattered plants of *Sarcocornia* spp. Some of these plants were dead and possibly remnants of vegetation from when the estuary had been hypersaline which was prior to heavy rains which occurred in November 1989. Grasses [*Sporobolus virginicus* Kunth., *Paspalidium obtusifolium* (Del.) Simpson, *Cynodon dactylon* (L.) Pers. and *Stenotaphrum secundatum* Kuntze] were found bordering the *Sarcocornia* spp. community as well as scattered along the length of the estuary. Isolated patches of *Phragmites australis* occurred along the whole length of the estuary, specifically at the confluence of small streams and seeps. These presumably represent sites of freshwater seepage.

Kromme river estuary

By contrast with the Gamtoos estuary, there was no spatial separation of macrophyte communities up the length of the Kromme estuary. This was associated with the absence of a salinity gradient. During the study period, salinities near the mouth ranged between 32 - 33.4 ppt, while in the upper reaches they ranged between 28.9 - 31.8 ppt. Apart from one salt marsh (salt marsh A, Fig. 5) which was dominated by *Spartina maritima* (Curtis) Fernald, the rest were dominated by *Sarcocornia decumbens*. The dominant submerged macrophyte, *Zostera capensis* Setchell, extended from the lower to the upper estuarine reaches (Fig. 5). *Phragmites australis* occurred at points along the estuary where streams entered the system. *Caulerpa filiformis* was the dominant macroalga occurring throughout the estuary (Fig. 5). Other macroalgae found in the Kromme estuary included: *Codium tenue* Kutz., *Gracilaria verrucosa* (Hudson) Papenfuss, *Hypnea viridis* Papenfuss, *Ulva rigida* C. Agardh, *Enteromorpha sp.*, *Colpomenia sp.* and *Ectocarpus sp.*. These were not associated with any particular position or environmental variable other than salinity and calm conditions. Consistently high water column salinities in the Kromme estuary have resulted in the accumulation of salts in the intertidal region. Recorded high sediment salinities (> 45 g g^{-1}) showed a negative association with macrophyte growth as no plants occurred at these sites.

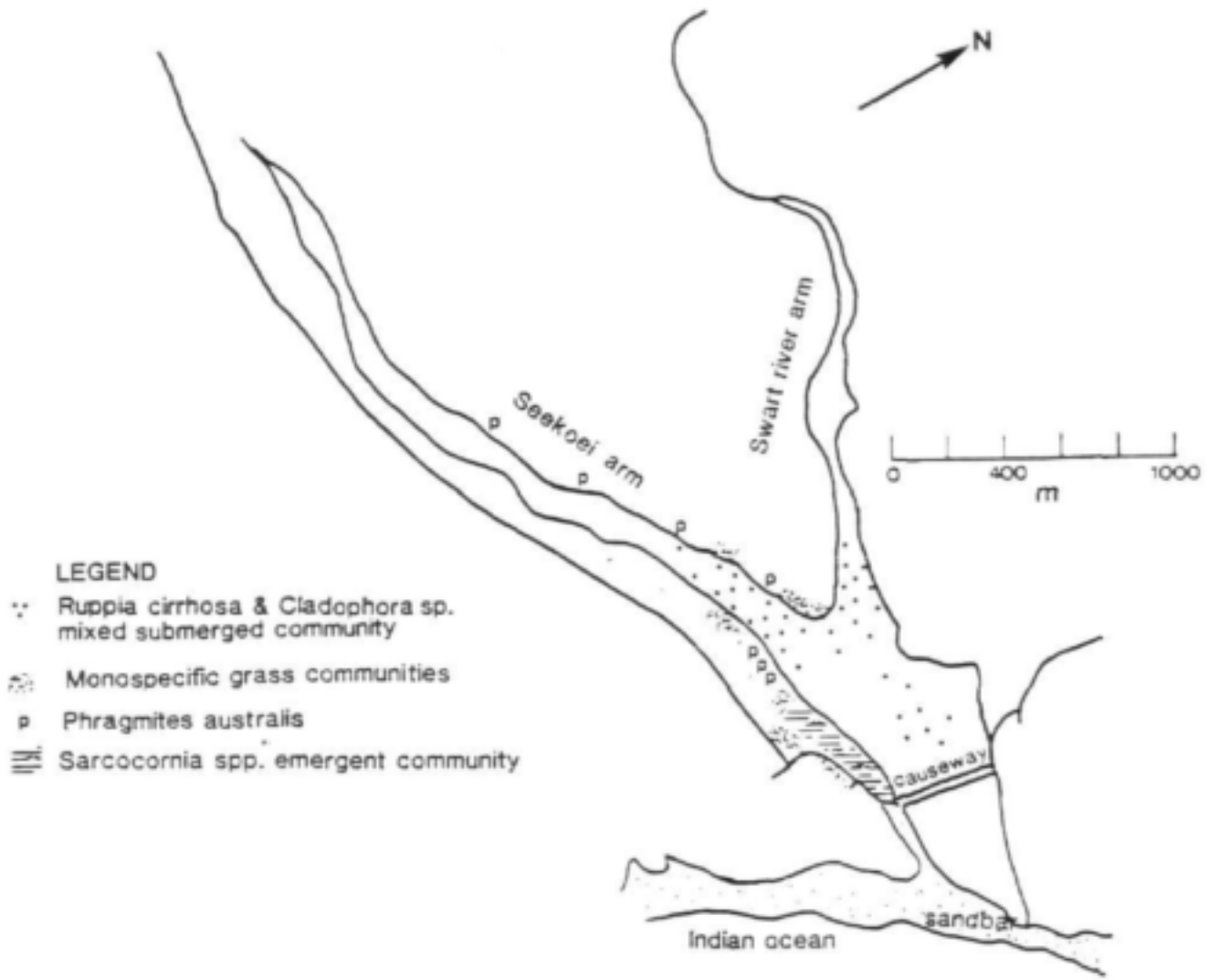


Figure 4 The distribution of macrophytes up the length of the Seekoei estuary.

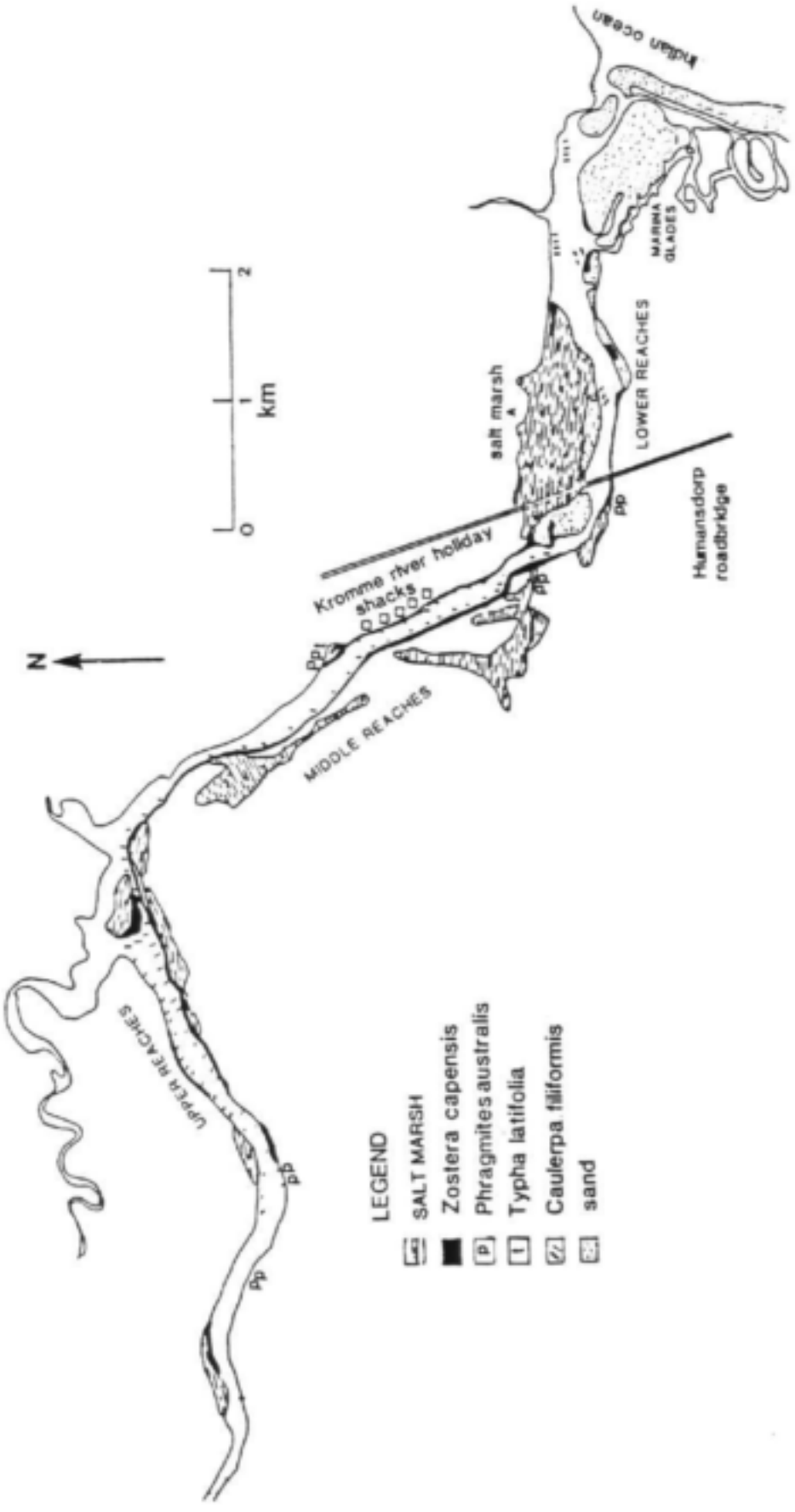


Figure 5 The distribution of macrophytes up the length of the Kromme estuary.

Environmental factors and community pattern (Ordination)

The ordination diagram in Figure 6 shows the distribution of macrophytes in all four estuaries in relation to the environmental data. The correlation coefficients (Table 2) indicated that the first axis is defined by an elevation gradient and the second axis by a salinity gradient. The macrophytes were found distributed along the elevation gradient, divided into three distinct communities: submerged, intertidal and supratidal communities (Fig. 6). Submerged macrophytes were dominant in the Kabeljous, Seekoei and Kromme estuaries. The majority of sites of these estuaries were therefore gathered at the lower left side of the ordination diagram (Fig. 6). Gamtoos sites were distributed throughout the ordination diagram, showing no distinct pattern but indicating the diversity of these sites and variety of macrophytes in this system. Salinity was also important in influencing distribution. Intertidal macrophytes dominant in the Gamtoos estuary (*Scripus maritimus*, *Phragmites australis*, *Cotula coronopifolia* L. and *Typha latifolia* L. subsp. *capensis* Rohrb.) occurred at the negative (brackish) end of the salinity gradient. Intertidal macrophytes dominant in the Kromme estuary (*Sarcocornia perennis* (Miller) Scott, *Chenolea diffusa* Thunb. and *Spartina maritima*) occurred towards the positive more saline end.

Table 2 Correlations coefficients between the environmental variables and axes 1 and 2 from the DCCA run, including sites and species from all four estuaries.

	AXIS 1	AXIS 2
ELEVATION	0.93	-0.10
SEDIMENT SALINITY (0-30 mm)	0.04	0.43
SEDIMENT SALINITY (30-70 mm)	0.06	0.46
SEDIMENT TOTAL PHOSPHORUS (0-30 mm)	0.21	0.01
SEDIMENT TOTAL PHOSPHORUS (30-70mm)	0.20	0.19
SEDIMENT AVAILABLE PHOSPHORUS (0-30 mm)	0.56	-0.02
SEDIMENT AVAILABLE PHOSPHORUS (30-70 mm)	0.55	0.29

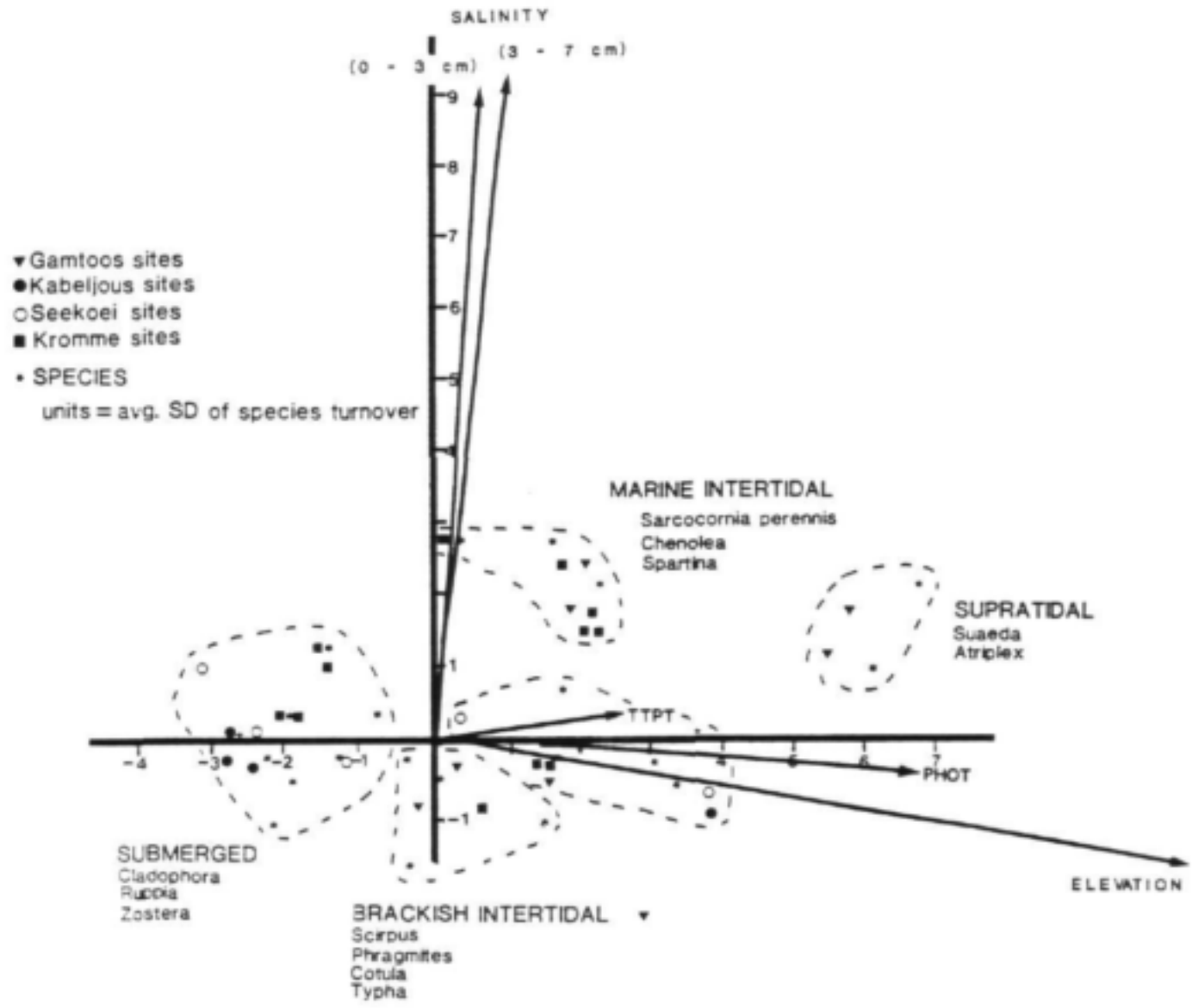


Figure 6 DCCA ordination diagram for all species and sites, first axis is horizontal and second axis is vertical. Environmental variable abbreviations are: TTPT = sediment total phosphorous concentration (0-30 mm), PHOT = sediment available phosphorous concentration (0-30 mm).

Community classification (TWINSPAN)

In order to assess whether the estuaries could be separated according to their emergent and submerged communities, sites were divided into emergent and submerged sites and classified separately. Figure 7, the dendogram for the submerged sites indicates that submerged sites can be divided into a Kromme marine group with *Zostera capensis* and *Gracilaria verrucosa* as diagnostic species and a Kabeljous and Seekoei brackish water group with *Ruppia cirrhosa* and *Cladophora sp.* as diagnostic species. Gamtoos estuary sites were included with the Seekoei and Kabeljous estuary group because none of these estuaries had *Z. capensis*. TWINSPAN separated the submerged macrophytes into two groups because they occur in different salinity regimes. The Kromme group comprises a marine community with salinities varying between 22 - 43 g g^{-1} while the Kabeljous and Seekoei group comprises a brackish community with salinities varying between 18 - 23 g g^{-1}.

The dendogram in Figure 8 indicates that these estuaries cannot be divided according to their emergent macrophyte communities. While certain species were only sampled in one system eg. *Juncus kraussii* Hochst in the Kabeljous estuary and *Spartina maritima* in the Kromme estuary, the majority of emergent macrophytes (eg. the *Sarcocornia* dominated community) were common to all four estuaries. Ubiquitous species included *Phragmites australis*, *Sarcocornia* spp. and *Sporobolus virginicus* Kunth.

The species diversity and the extent of emergent communities was related to topography and available physical space. The Shannon-Weaver diversity index (H') was therefore higher for the larger Gamtoos (H' = 0.995) and Kromme (H' = 1.017) estuaries than for the smaller Seekoei (H' = 0.708) and Kabeljous (H' = 0.792) estuaries.

Salinity ranges for individual macrophytes

Salinity ranges for macrophytes from the four estuaries were pooled in order to present the widest range of salinity tolerance for the individual macrophytes. For the intertidal macrophytes, the salinity of the top sediment layer was generally higher than that of the bottom (Fig. 9). Water contents followed this same pattern (Fig. 9), indicating the role the adjacent water body plays in maintaining sediment salinities in the intertidal sites.

Of all the intertidal macrophytes (Fig. 9) *Cotula coronopifolia* was found in the lowest salinities. It occurred in the brackish upper reaches of the Gamtoos estuary. Although *Scirpus maritimus* and *Phragmites australis* are usually regarded as freshwater emergent macrophytes (Benfield 1984, Bally *et al.* 1985) they were found in this study in polyhaline (18 - 30 ppt) to euhaline (30 - 40 ppt) conditions.

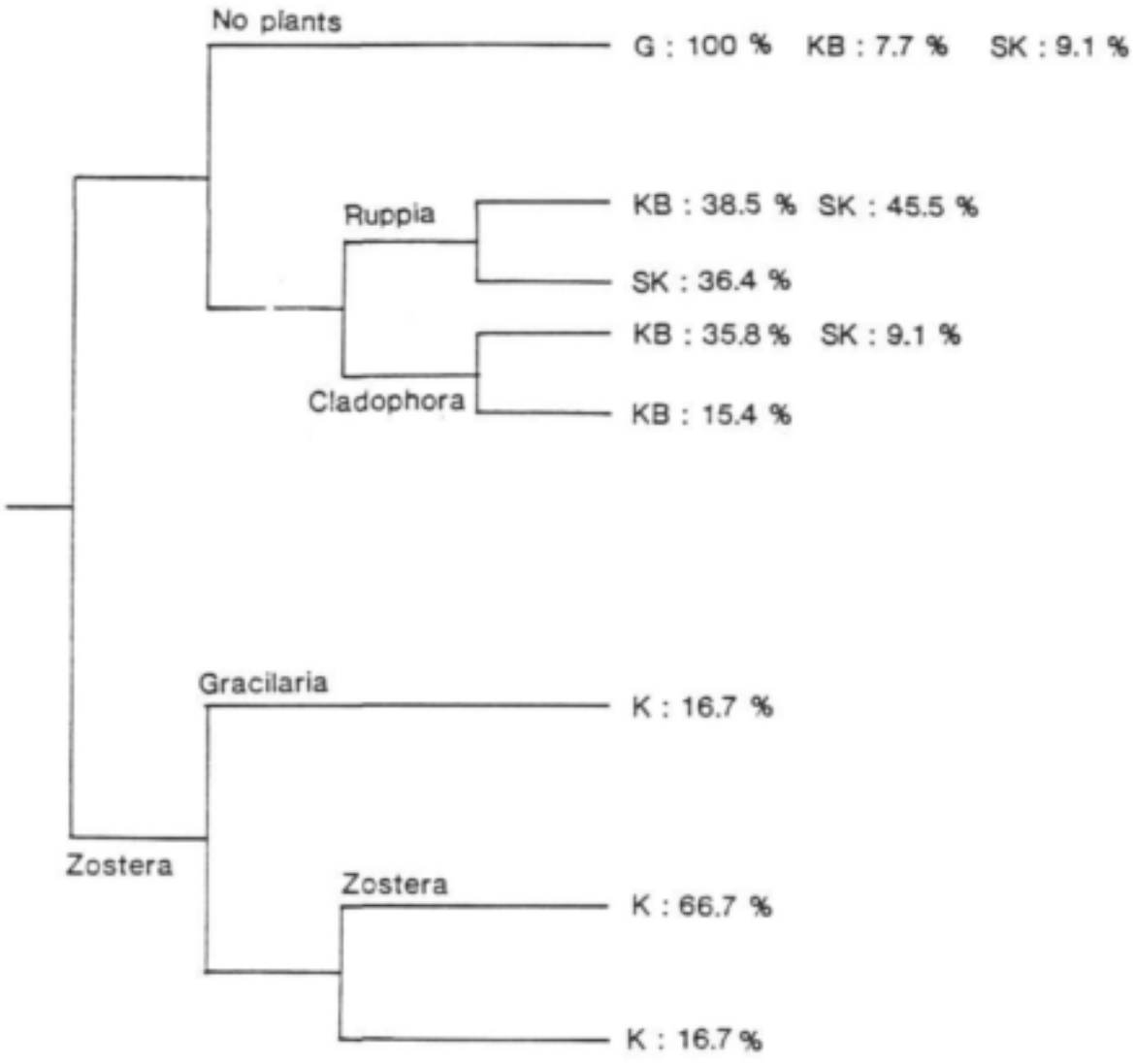


Figure 7 Twinspan classification dendogram for all submerged sites. Estuary abbreviations are: G = Gamtoos, KB = Kabeljous, SK = Seekoei and K = Kromme.

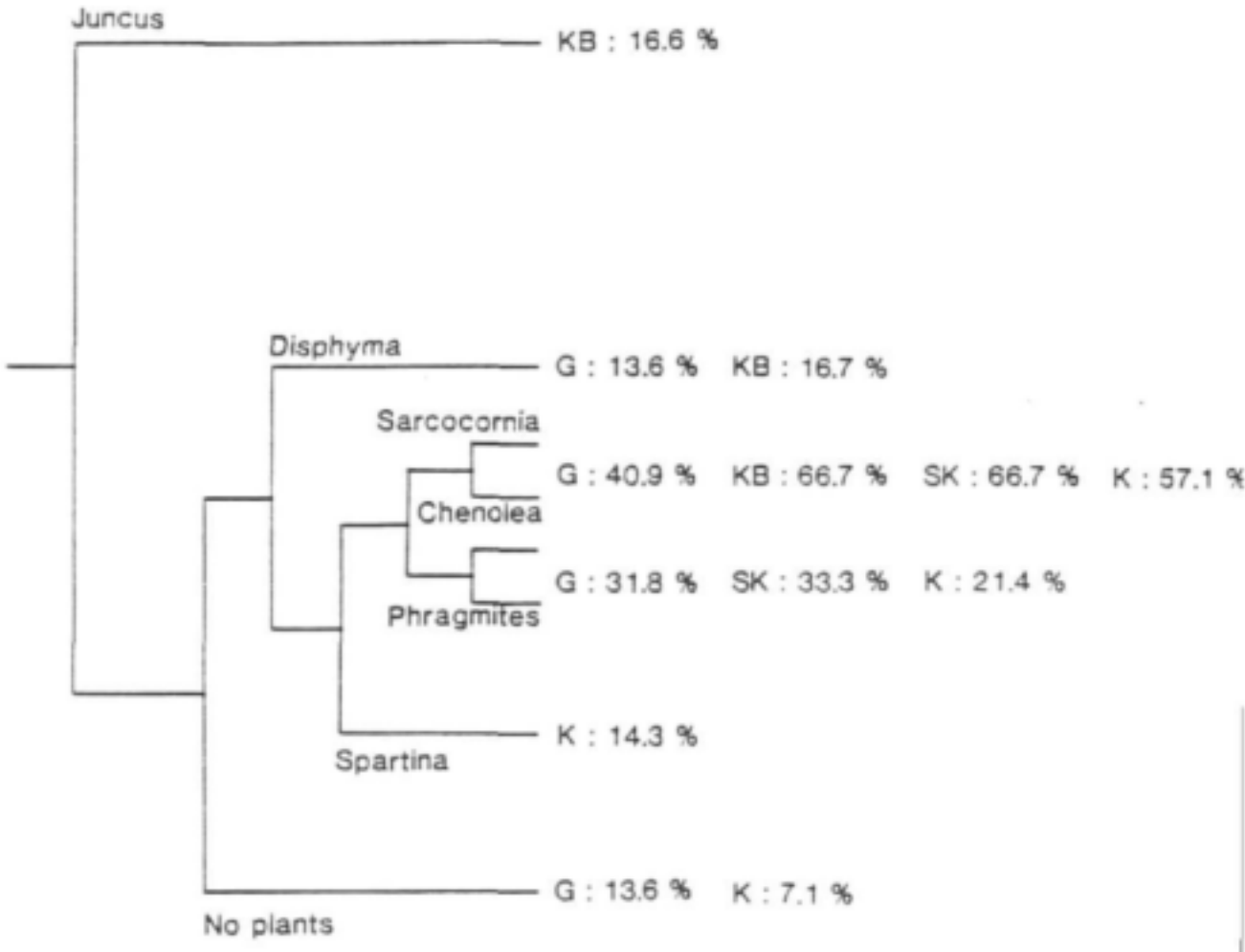


Figure 8 Twinspan classification dendogram for all emergent sites. Estuary abbreviations are. G = Gamtoos, KB = Kabeljous, SK = Seekoei and K = Kromme.

The supratidal macrophytes *Disphyma crassifolium*, *Juncus kraussii*, *Suaeda fructicosa* and *Atriplex sp.* were associated with lower sediment salinities and sediment water contents than the intertidal species (Fig. 10). Tidal flushing of these areas is limited, as was indicated by the low water contents of the sediments associated with these species (Fig. 10). Sediment salinities for these species were also lower than the salinities of the adjacent estuarine water body. The salinity of the supratidal sediments is possibly controlled more by rainfall and evaporation. By contrast, the supratidal species *Sarcocornia pillansii* (Moss) Scott occurred in sediments with salinities ranging between 30 - 48 g g^{-1}, i.e. similar to those of the most saline intertidal sites. *Sarcocornia pillansii* possibly tolerates higher salinities than other supratidal macrophytes.

Discussion

The macrophyte flora was compared in four estuaries with different physical characteristics. Estuaries ranged from small to large, from periodically closed systems to systems with permanently open mouths and good tidal exchange; from estuaries with no freshwater input to estuaries with continual freshwater inflow. The role of freshwater in estuaries today differs from what it would have been in the past when river catchments were free of dams and industrial and agricultural pollution was absent. Cause and effect phenomena between freshwater and macrophytes cannot be deduced directly, but associations between macrophytes and the salinity (freshwater input) status of estuaries, as they are today, can be identified.

Continual freshwater input in the Gamtoos estuary created a salinity gradient from the mouth to the head of the estuary along which macrophytes were distributed. In contrast, spatial separation of macrophytes up the length of the Kromme estuary was absent. This is believed to be due to the lack of freshwater input which caused uniformly high salinities to occur throughout the estuary. These high salinities increased marine macroalgal colonization from the sea and allowed *Zostera capensis* to extend into the upper reaches of the estuary. Dense brackish macrophyte communities consisting of reeds, *Phragmites australis* and *Scirpus maritimus* and submerged macrophytes, *Potamogeton pectinatus* and *Ruppia sp.*, were absent from the upper reaches. These communities are present in other estuaries which have longitudinal salinity gradients e.g. Swartvlei estuary on the southern coast of South Africa (Howard-Williams and Liptrot 1980).

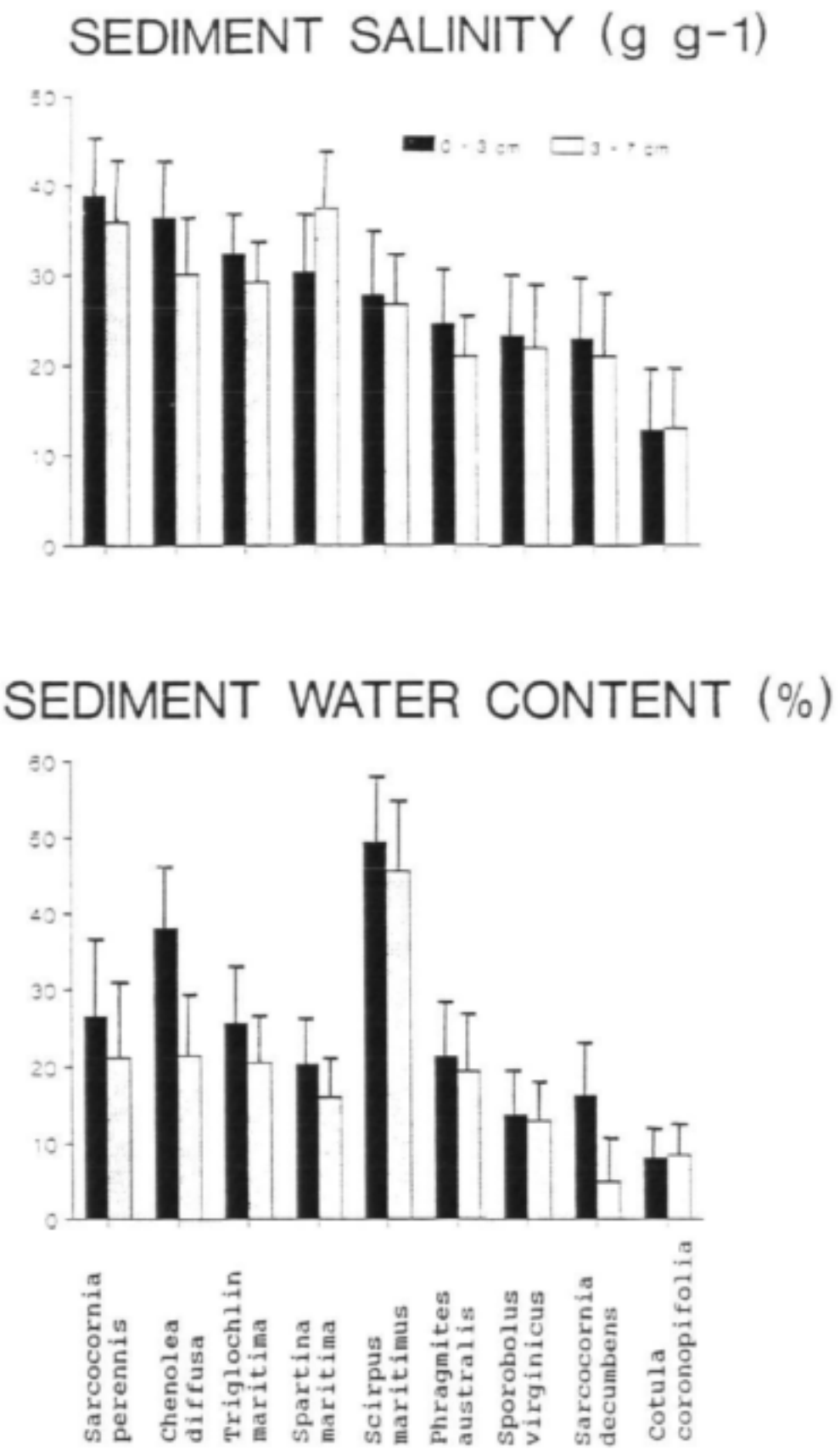


Figure 9 Sediment salinities and water content (means and ranges, n = 6) for dominant intertidal emergent macrophytes (top sediment layer 0-3 cm, bottom sediment layer 3-7 cm).

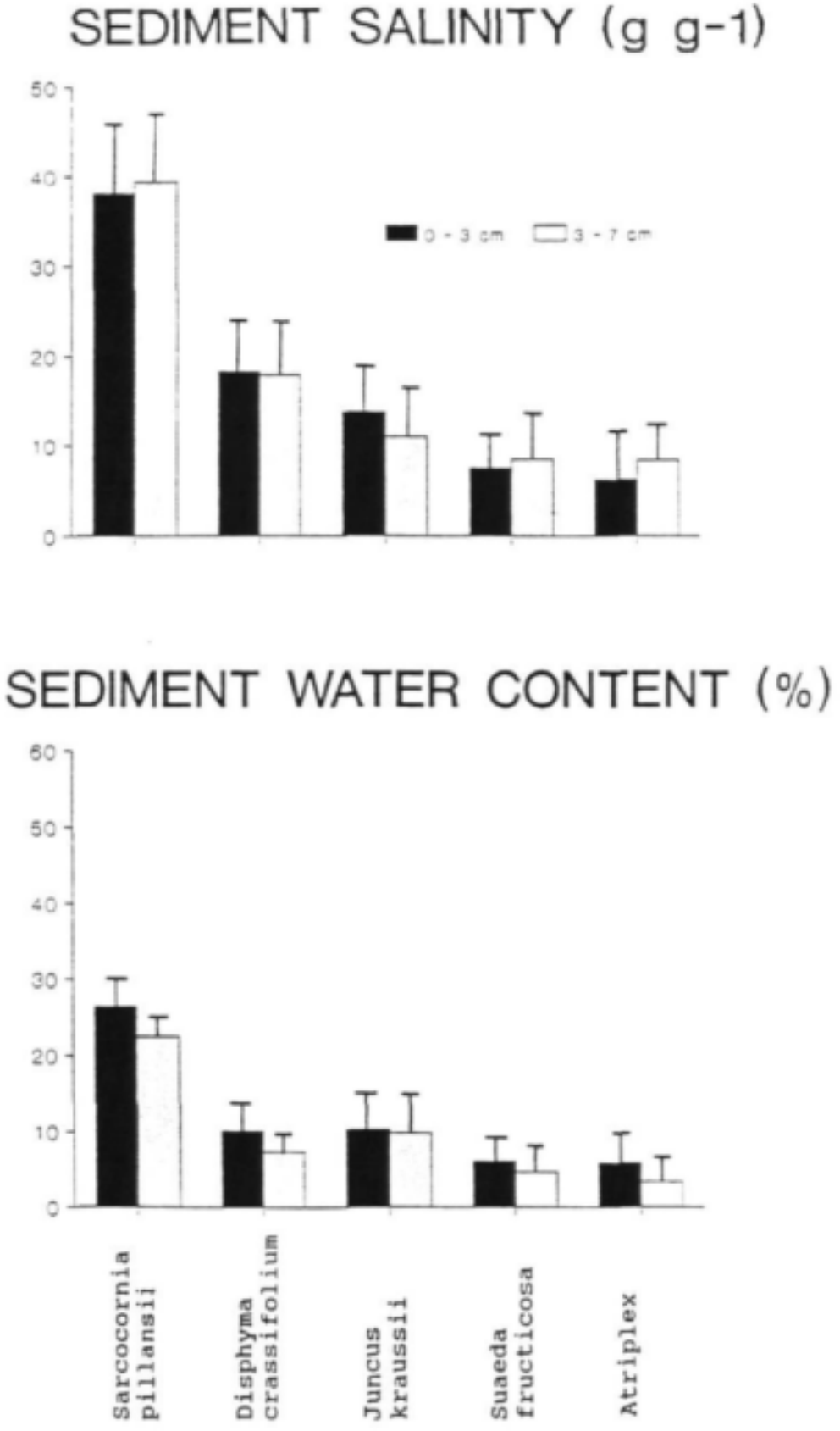


Figure 10 Sediment salinities and water content (means and ranges, n = 6) for dominant supratidal emergent macrophytes (top sediment layer 0-3 cm, bottom sediment layer 3-7 cm).

Despite the lack of distinct zonation up the length of the Kromme estuary, some variation did occur. *Phragmites australis* occurred at the confluence of small streams and seeps. Such outcrops are characteristic of freshwater seepage into marine lagoons (Christie 1981). Data on runoff below impoundments and groundwater seepage into estuaries is lacking. It is important that this is quantified, as freshwater input from these localized sources may prevent hypersaline conditions and impoverishment of an estuary during times of drought. The contribution of freshwater inputs from such point sources may acquire great importance in the future and should be borne in mind whenever small farm dams are constructed.

The lack of freshwater input into the Kromme estuary was not only associated with a lack of spatial separation of macrophytes up its length, but was also associated with a reduction in the temporal dynamics of macrophyte communities. Fluctuations in *Z. capensis* biomass in response to flooding (Talbot *et al.* 1990, Hanekom and Baird 1988) are attenuated as the dams in the catchment prevent all floods smaller than a 1-in-30 year event (Fromme and Badenhorst 1987). *Zostera capensis* biomass and cover in the Kromme estuary has increased since the construction of the second dam due to reduced flooding and increased sediment stability in the system (Adams and Talbot 1992).

Submerged communites were dominant in the small Kabeljous and Seekoei estuaries and in the marine-dominated Kromme estuary. Salinities in the smaller estuaries fluctuate widely because of the greater effect of periodic small floods. In 1990, rainfall was adequate to maintain brackish conditions (13-28 ppt) in these small estuaries. Submerged macrophyte communities were therefore polyhaline (18 - 30 ppt) with rooted *Ruppia cirrhosa* and the green alga *Cladophora sp.* as the dominant genera. *Ruppia* spp. are restricted to sheltered habitats because of their vegetative structure. The thin stems lack supporting tissue and the rooting parts are poorly developed (Verhoeven 1979). *Ruppia* is thus known to thrive in systems where water currents are low and sediments finely grained (Congdon and McComb 1979). Hence they occurred in the periodically closed Kabeljous and Seekoei estuaries, not only because of the low salinities but also because of reduced water velocities.

Although submerged macrophytes were found towards the mouth and head of the Gamtoos estuary, they were absent along its greatest length. This can be attributed to physical features such as depth and high turbidities caused by a strong freshwater input. Consistent freshwater inflow transports suspended sediments and debris which increases water column turbidity and reduces light available to submerged macrophytes. Light availability is most commonly cited as the environmental factor limiting submerged macrophyte distribution (Spence 1981).

The estuaries studied could be separated according to their submerged macrophyte communities, but because the majority of emergent macrophytes were found in all four estuaries they could not be separated according to their emergent macrophyte communities.

Species diversity and the extent of emergent communities appears to be related to topography and available physical space. Species diversity was higher in the larger Gamtoos and Kromme estuaries as there is more spatial heterogeneity and therefore more habitats are available for colonization. *Spartina maritima* occurred exclusively in the Kromme estuary. According to Day (1981a), *S. maritima* is absent from most blind estuaries as growth is dependent on frequent tidal exchange. This would explain its absence from the periodically blocked Kabeljous and Seekoei estuaries. *Phragmites australis* was ubiquitous in all four estuaries possibly because it consists of a wide range of ecotypes (Björk 1967).

The low diversity and limited emergent macrophyte cover in the small estuaries can be related to temporal variation including fluctuating water levels and periodic hypersaline conditions. In the Seekoei estuary the causeway influences species composition and diversity as it permanently raises water levels in the upper estuary and causes macrophytes to be inundated for long periods of time. Grasses are the dominant emergent macrophytes, because species such as *Sporobolus virginicus* grow well under waterlogged conditions and can withstand long periods of submergence (Breen *et al.* 1977). High salinities result from lack of freshwater input into the system during times of drought. Before the floods of November 1989, salinities greater than 90 ppt were recorded on the landward side of the causeway in the Seekoei estuary. Few salt marsh species will germinate at salinities greater than 20 ppt (Chapman 1976) and productivity (Barbour and Davis 1970, and Price *et al.* 1988), growth, density and species diversity (Ungar 1974) are reduced at high salinities.

Supratidal communities were characterized by low salinities and low sediment water contents as these communities were found in higher less frequently flooded areas. It has been shown that salinity decreases with increasing elevation and decreased tidal flushing (Bertness and Ellison 1987 and Adam 1981). Indeed certain supratidal species such as *Suaeda* are intolerant of waterlogging (Walsh 1974). By contrast sediment salinities of the intertidal macrophyte communities were determined by the salinity of the adjacent water body at all times. High water column salinities in the Kromme estuary resulted in the accumulation of salts in the intertidal zone. No plants occurred at these salinities and salt marshes in these areas were characterized by numerous bare sandy patches. Future research should aim at determining the role of rainfall and tidal inundation in regulating salt marsh salinities because a lack of freshwater flooding could result in hypersaline conditions in salt marshes which may cause a threat to the estuarine environments as we know them today.

3. THE FRESHWATER REQUIREMENTS OF ESTUARINE MACROPHYTES

3.1 SUBMERGED MACROPHYTES

3.1.1 THE EFFECT OF SALINITY ON *RUPPIA CIRRHOSA* (PETAGNA) GRANDE AND *ZOSTERA CAPENSIS* SETCHELL.

Introduction

The flow of freshwater into South African estuaries is becoming restricted to the extent that it is expected to threaten submerged macrophyte communities. High salinity and fluctuating water levels resulting from erratic freshwater input has resulted in the exposure and dieback of submerged communities. *Ruppia cirrhosa* (Petagna) Grande (Plate 1) and *Zostera capensis* Setchell (Plate 2) are dominant and key submerged macrophytes in many South African estuaries. They provide a substantial amount of primary productivity, nutrient storage and nursery habitats in shallow estuarine waters. *Ruppia cirrhosa* is common under brackish conditions while *Z. capensis* is common in more saline conditions (Day 1981b, Howard-Williams and Liptrot 1980).

To study the relationship between the two dominant species, the long term effects of salinity on *Z. capensis* and *R. cirrhosa* were determined from controlled laboratory experiments. The tolerance to salinity *in situ* of these submerged macrophytes was also determined from surveys of local estuaries. Proline accumulation in *R. cirrhosa* and *Z. capensis* roots and leaves was measured in plants grown at different salinity levels. It is thought that the ability to tolerate high salinity is linked to the accumulation of this compatible solute (Brock 1981a). A comparison of growth strategies and the co-existence of the plants under the same salinity conditions was studied. Studies were also conducted to determine how the plants regrow or re-establish, after exposure to hypersaline conditions. Germination of *R. cirrhosa* seeds at different salinity levels was investigated.

Methods

Salinity Tolerance

Plants were collected from the field and allowed to adapt to growth cabinet conditions for one week before being placed in separate tanks. The tanks were placed in a controlled environmental chamber (Conviron, Winnipeg) where fluorescent tubes and incandescent bulbs provided 550 - 700 $\mu E\ m^{-2}\ s^{-1}$ photosynthetic active radiation in a 12:12 light:dark cycle. Temperature was maintained at 20 °C.

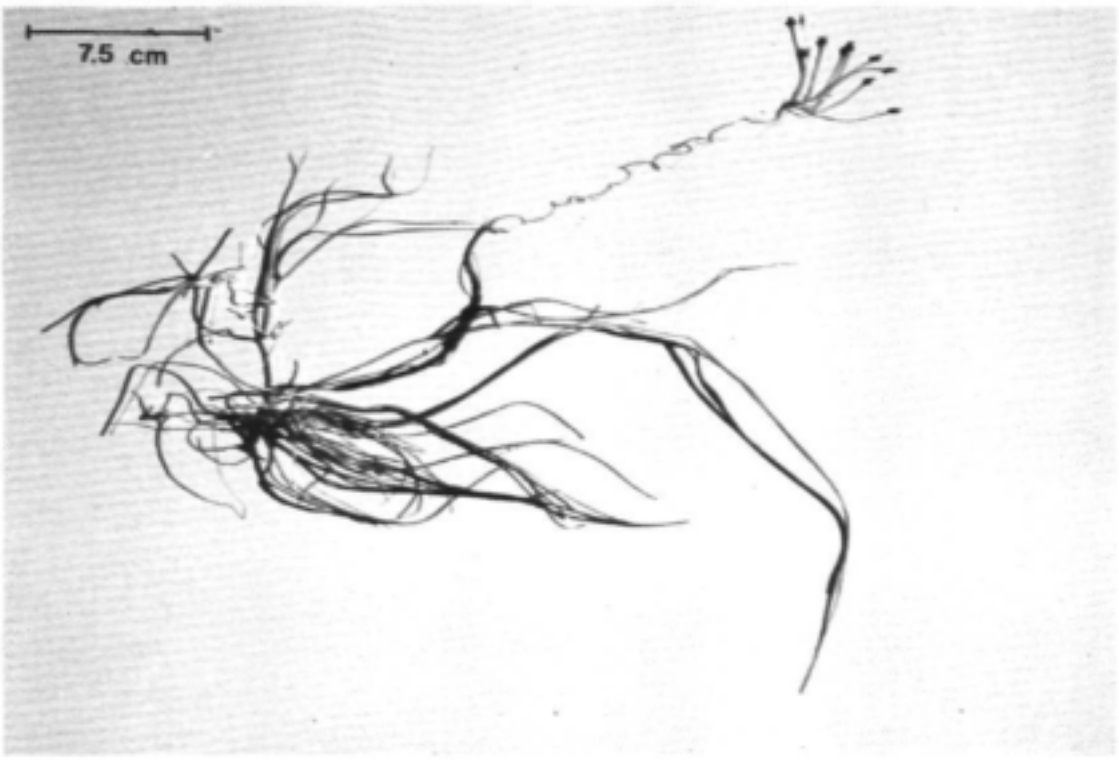


Plate 1 *Ruppia cirrhosa* (Petagna) Grande.

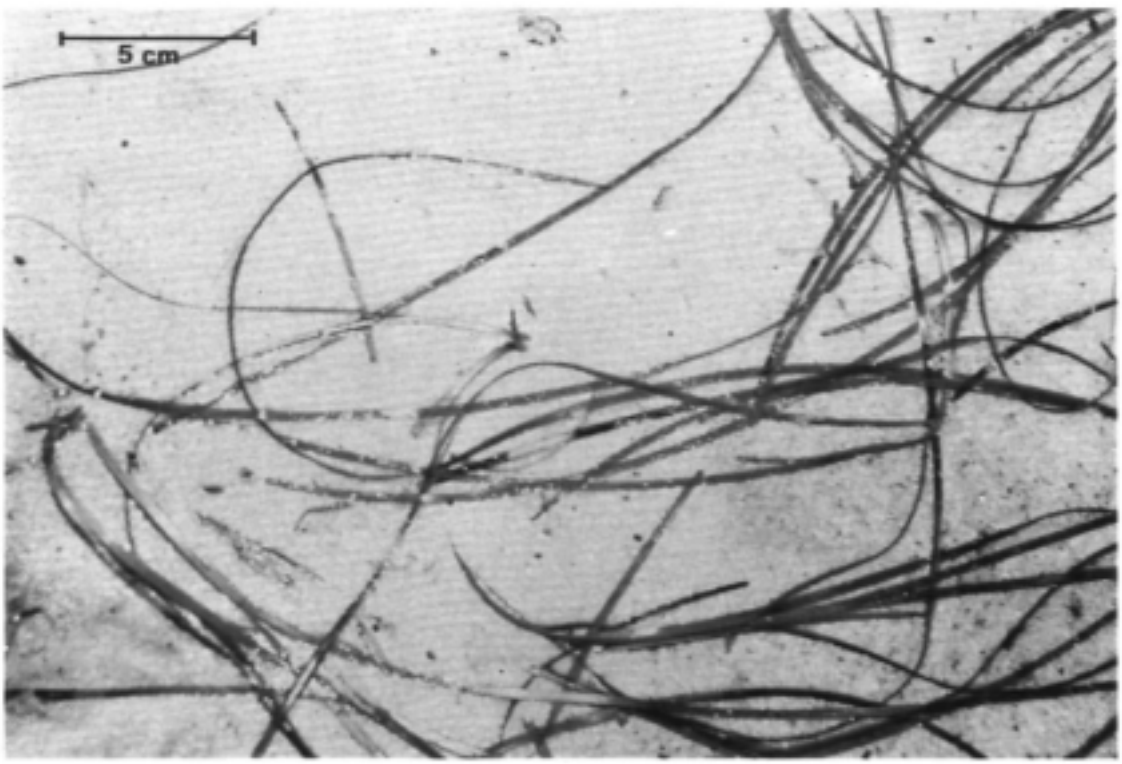


Plate 2 *Zostera capensis* Setchell.

A separate tank was then prepared for each salinity treatment. Each held approximately 17 l of water and contained 12 plants of approximately equivalent size and having shoots and attached roots. Initial wet weight, number of leaves, rhizome and leaf length was measured for each plant. Submerged macrophytes were planted in sediment obtained from the habitat where they were collected. The tank was separated into larger and smaller sections by a perspex sheet. The larger section contained the sediment and rooted submerged macrophytes and the smaller section contained an airlift system and filter that circulated and aerated the water through the rest of the tank. This two-part tank prevented the resuspension of sediment when the tank was topped up or refilled with water.

Survival of the submerged macrophytes was determined over a three month period at 5 salinity treatments: 0, 15, 35, 55 and 75 ppt. Hypersaline media were obtained by adding coarse sea salts (National Ingredients Ltd., Port Elizabeth) to filtered seawater. Hyposaline media were obtained by diluting filtered seawater with distilled water.

Salinity was checked twice weekly with an Atago S/Mill hand refractometer (Labotec) and adjusted as required. The water in the tanks was completely replenished every week and epiphytes were removed by gently wiping the plant surfaces with soft tissue and wiping the sides of the tanks.

An assessment was made each week of the plants. Weekly assessments were made and plants were assessed as 'dead' when either all leaves had been shed or the leaves were blackened. After three months the plants were harvested and wet mass, average maximum leaf length, rhizome length and number of leaves per plant was measured. The plants were blotted dry with absorbent paper to remove excess water before wet mass was measured.

A survey of estuaries along the Cape Province coastline was undertaken to determine the distribution of *Z. capensis* and *R. cirrhosa* in relation to natural salinity conditions. Altogether, 22 estuaries were visited as shown in Figure 11.

Competition and Proline Accumulation

Similarly sized plants of *Z. capensis* and *R. cirrhosa* (4 replicates), were grown on opposite sides of the same tank. Tanks were made up with 0, 15, 35 and 55 ppt water. Initial wet biomass, leaf and rhizome length was measured for each plant. The condition of the plants and the number of leaves in the tank was monitored over a period of 5 months. Plants were then harvested and proline concentrations and various parameters measured and compared for each of the two species.

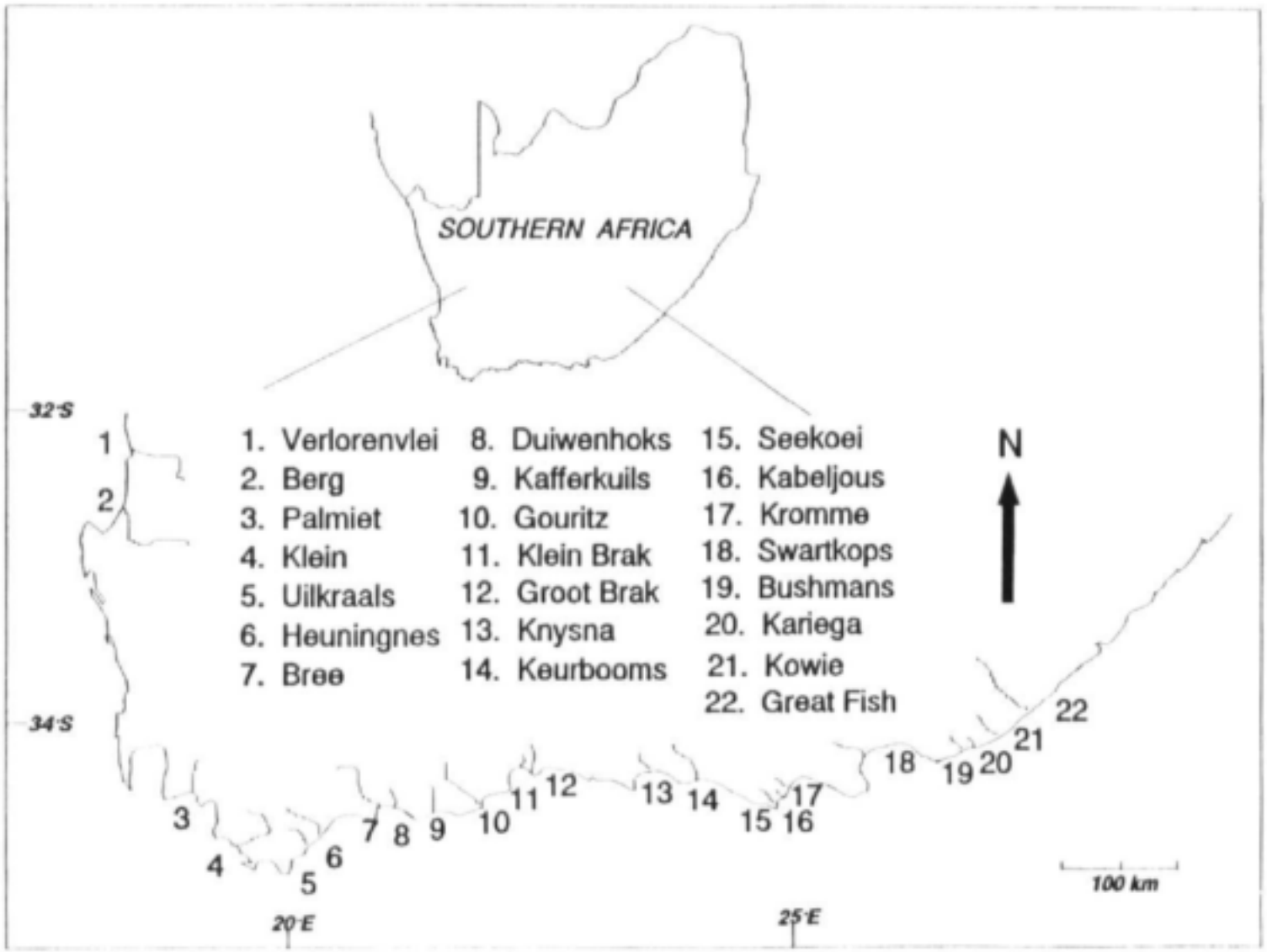


Figure 11 Map of southern Africa showing the localities of the estuaries sampled.

Proline was extracted from 0.5 g freeze-dried root and leaf samples ground up with 10 ml of 3 % sulfosalicyclic acid and determined according to the method of Bates *et al.* (1973). Proline concentrations were expressed as μmoles proline/g fresh mass. The data were subjected to an analysis of variance using the SOLO statistical package (BMDP Statistical Software, Inc. 1988). When significant F-values were detected, means were separated by the Student Newman-Keuls multiple range test.

Recovery from a High Salinity Treatment

In a separate experiment, 10 replicates each of *R. cirrhosa* and *Z. capensis* were placed in separate 75 ppt treatment tanks. The *R. cirrhosa* control tank was maintained at 0 ppt while the *Z. capensis* control tank was maintained at 15 ppt. The reason for this was that the previous salinity tolerance experiment had shown these to be their optimum growth salinity. Plants were exposed to a hypersaline treatment of 75 ppt until over 80 % of the leaf tissue was chlorotic or dead. Salinity was then gradually reduced each week and the recovery of the plants monitored by counting the number of leaves in each tank. The increase in the number of leaves over time was compared for the different plants and the controls.

Seed Germination

Germination of *R. cirrhosa* seeds in different salinity treatments was also studied. Fifty seeds in each test were placed in petri dishes with solutions of different salinity and germination was recorded on a daily basis. Germination of *Z. capensis* could not be studied as insufficient seeds were available.

Results

Salinity tolerance

The wet mass of *Z. capensis* (final - initial), rhizome length and number of leaves (final - initial) (Figs. 12 A, B, C) were all maximal when grown at a salinity of 15 ppt. Leaf length was greatest at 35 ppt but was not significantly different ($P > 0.05$) from 15 ppt (Fig. 12D). *Zostera capensis*, therefore, appears to survive best within the salinity range of 15 and 35 ppt. The change in wet mass of *Z. capensis*, the number of leaves as well as the rhizome and leaf lengths (Fig. 12) were higher in freshwater than in hypersaline treatments (55 - 75 ppt). It appears that *Z. capensis* would survive better under extended freshwater conditions than under extended hypersaline conditions. After only six weeks of incubation, the plants in freshwater (0 ppt) began to show signs of stress, whereas after four weeks of incubation in 75 ppt, all the *Z. capensis* plants had died. Plants in 55 ppt initially survived but, as time progressed, they

became increasingly stressed and had died after 12 weeks.

The change in wet mass of *R. cirrhosa*, rhizome length and number of leaves (Figs. 13 A, B, C) decreased as the treatment salinity increased from 0 to 75 ppt. Leaf length peaked at 35 ppt (Fig. 13D). Horizontal growth of *R. cirrhosa* was greater than vertical growth in the 0 and 15 ppt treatments. The plants were characterized by numerous creeping, branching rhizomes and many short leaves. Plants in the 35 ppt treatment had long vertical branching stems with fewer leaves of longer leaf length (Fig. 13D). Kiørboe (1980) also reported a lack of vertical growth in *R. cirrhosa* at low salinity (0 - 6 ppt). New growth was evident for all *R. cirrhosa* plants but was greatest at the lower salinity levels (Fig. 13D). Plants flowered in the salinity treatments from 0 - 55 ppt.

The survey of different estuaries showed that *Z. capensis* occurred over a wide range of salinity from 12 ppt in the Klein Brak estuary to 44 ppt in the Bushmans estuary. However, *Z. capensis* was dominant in marine estuaries (salinity between 30 - 40 ppt). *Ruppia cirrhosa*, on the other hand, was confined to temporarily closed brackish estuaries where salinity was below 30 ppt. *Ruppia cirrhosa* occurred in salinity ranging from 12 ppt in the Klein Brak estuary to 30 ppt in the Great Brak estuary. *Zostera capensis* and *R. cirrhosa* were found growing together in the Klein Brak estuary (salinity: 15 ppt), Klein estuary (salinity: 16 ppt) and Knysna estuary (salinity: 20-25 ppt). *Zostera capensis* always occurred at greater depths than did *R. cirrhosa*. The localities of the estuaries mentioned are shown in Figure 11.

Competition and proline accumulation

For the competition experiment plant growth parameters are expressed as totals for the tank and not averages for the 4 replicates. This is because after five months the plants had expanded extensively and it was not possible to identify individual replicates with certainty.

After five months *Z. capensis* had completely died in the 0 and 55 ppt salinity treatments. Therefore, competition between the species can only be compared in the treatments 15 and 35 ppt. In the previous salinity tolerance experiment, *Z. capensis* plants in 55 ppt died after 12 weeks. In this experiment *Z. capensis* was dead after 9 weeks in the 55 treatment. The plants were grown in the same tank and therefore competition for nutrients and space by *R. cirrhosa* may have affected the survival capacity of *Z. capensis* under hypersaline conditions.

Although both species were of equivalent size and mass at the beginning of the growth period, *Ruppia cirrhosa* expanded and grew more than did *Z. capensis* in 15 ppt. The change in total rhizome length (Fig. 14A), number of leaves (Fig. 14B) and wet biomass (Fig. 14C) for *R. cirrhosa* after 5 months of growth was higher than for *Z. capensis*. There was, however, no difference in final leaf length (Fig. 14D).

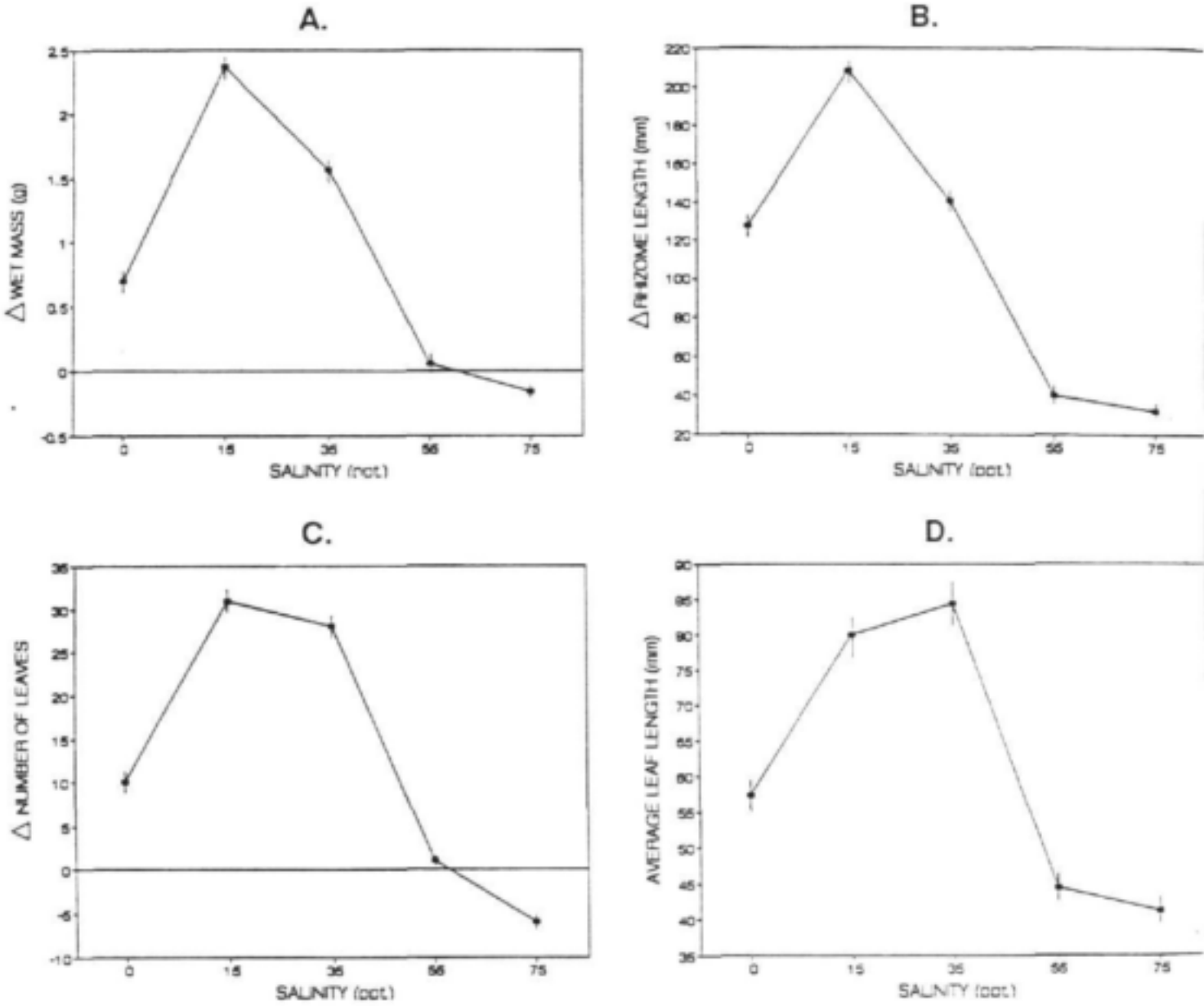


Figure 12 The effect of salinity on the change of *Zostera capensis* wet mass (A), rhizome length (B), number of leaves (C) and average leaf length (D). (Vertical bars = SE).

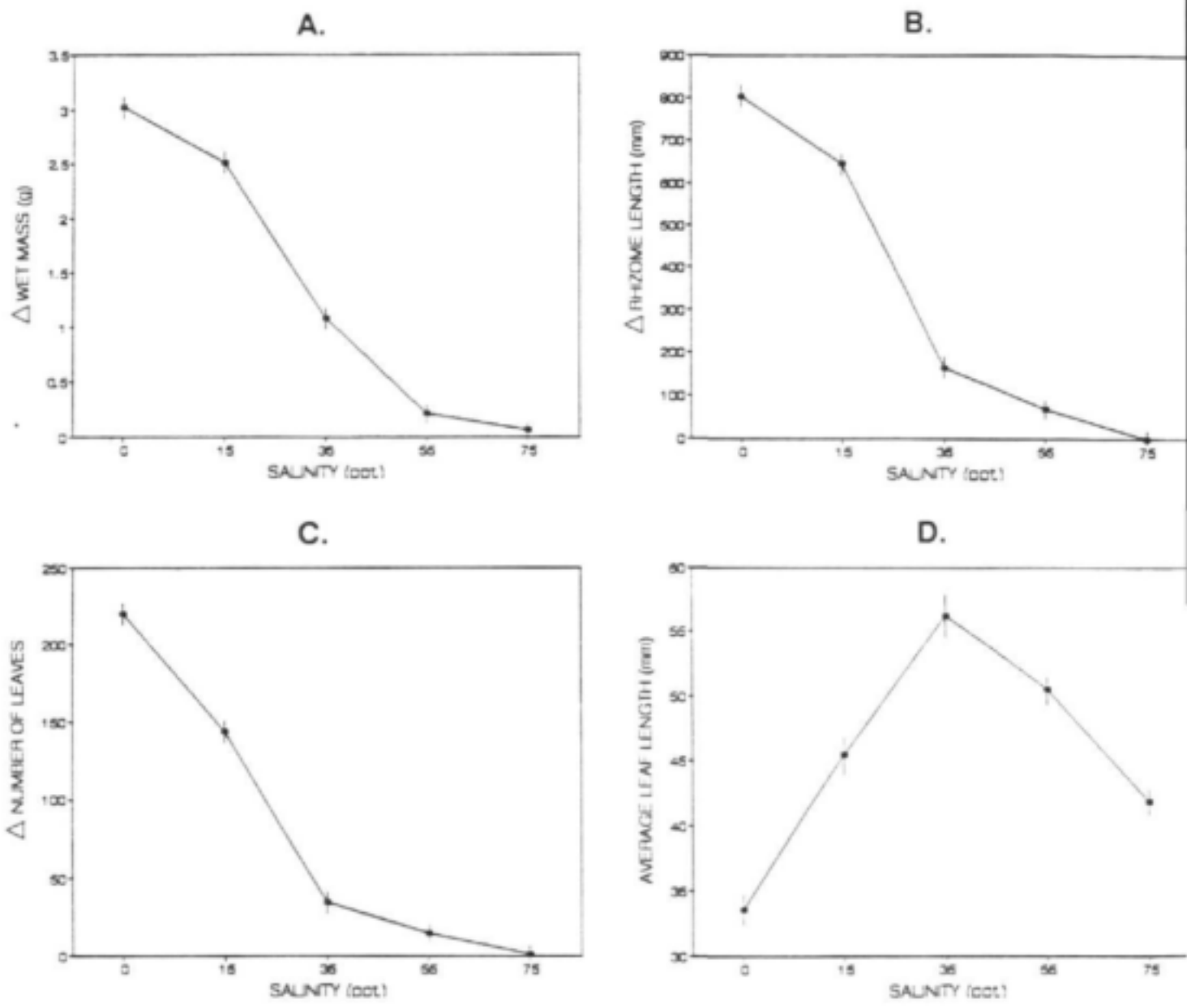


Figure 13 The effect of salinity on the change of *Ruppia cirrhosa* wet mass (A), rhizome length (B), number of leaves (C) and average leaf length (D).

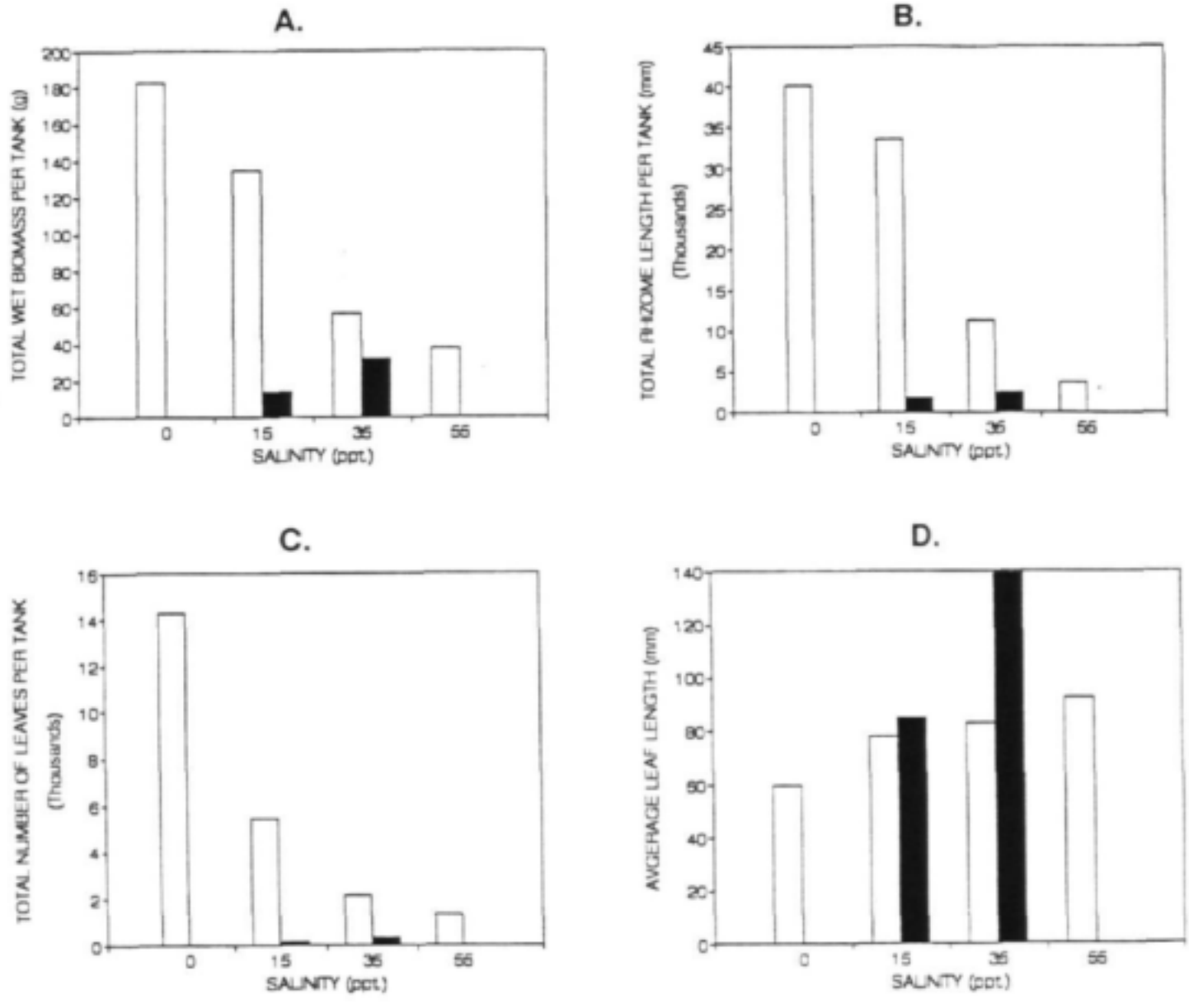


Figure 14 The wet biomass (A), total rhizome length (B), total number of leaves (C) and average leaf length (D) per tank for *Ruppia cirrhosa* (□) and *Zostera capensis* (■) after a 5 month period.

Proline concentrations in leaf and root tissue was significantly affected by salinity treatment ($F = 56.27$, $P < 0.0001$). Root proline concentrations were generally less than leaf concentrations (Table 3). Proline concentrations in *R. cirrhosa* leaf tissue was higher at higher external salinity. Mean leaf proline concentration at 55 ppt was significantly higher than at 35 ppt and significantly higher than the 15 ppt and freshwater treatments (Table 3).

Ruppia cirrhosa had significantly more proline in leaf material than did *Z. capensis* at 35 ppt (Table 3). At 15 ppt and less, leaf and root concentrations of proline were not significantly different. A separate analysis of variance for the *Z. capensis* data showed that the proline concentration in leaf and root tissue for the 35 ppt salinity treatment was significantly higher than in the 15 ppt treatment ($F = 6.33$, $P < 0.005$).

Table 3 The effect of salinity on proline accumulation (μmoles gram fresh mass^{-1}) in *R. cirrhosa* and *Z. capensis*. Means with different letters are significantly different at $P < 0.05$ using the Newman/Keuls range test. (NS = not sampled, refer to text).

Salinity	Leaves		Roots	
(ppt)	*Ruppia*	*Zostera*	*Ruppia*	*Zostera*
0	0.008^{a}	NS	0.006^{a}	NS
75/15	0.015^{a}	NS	0.011^{a}	NS
15	0.052^{ab}	0.036^{a}	0.009^{a}	0.021^{a}
35	0.179^{c}	0.084^{ab}	0.056^{ab}	0.079^{ab}
55	0.426^{d}	NS	0.112^{b}	NS

Recovery from a High Salinity Treatment

A reduction in water salinity resulted in an increase in the number of *R. cirrhosa* leaves (Fig. 15). In the control tank (0 ppt), the number of leaves increased exponentially with time (Fig. 15). Only four data points are shown in the figure as the number of leaves became too numerous to count. When the tanks were harvested there were 7803 leaves in the control tank. *Zostera capensis* did not recover when salinity was reduced. When the tanks were harvested only dead rhizome portions of *Z. capensis* could be found. There were no leaves as they had been abscised. For the 15 ppt control tank the number of leaves increased over time (Fig. 15).

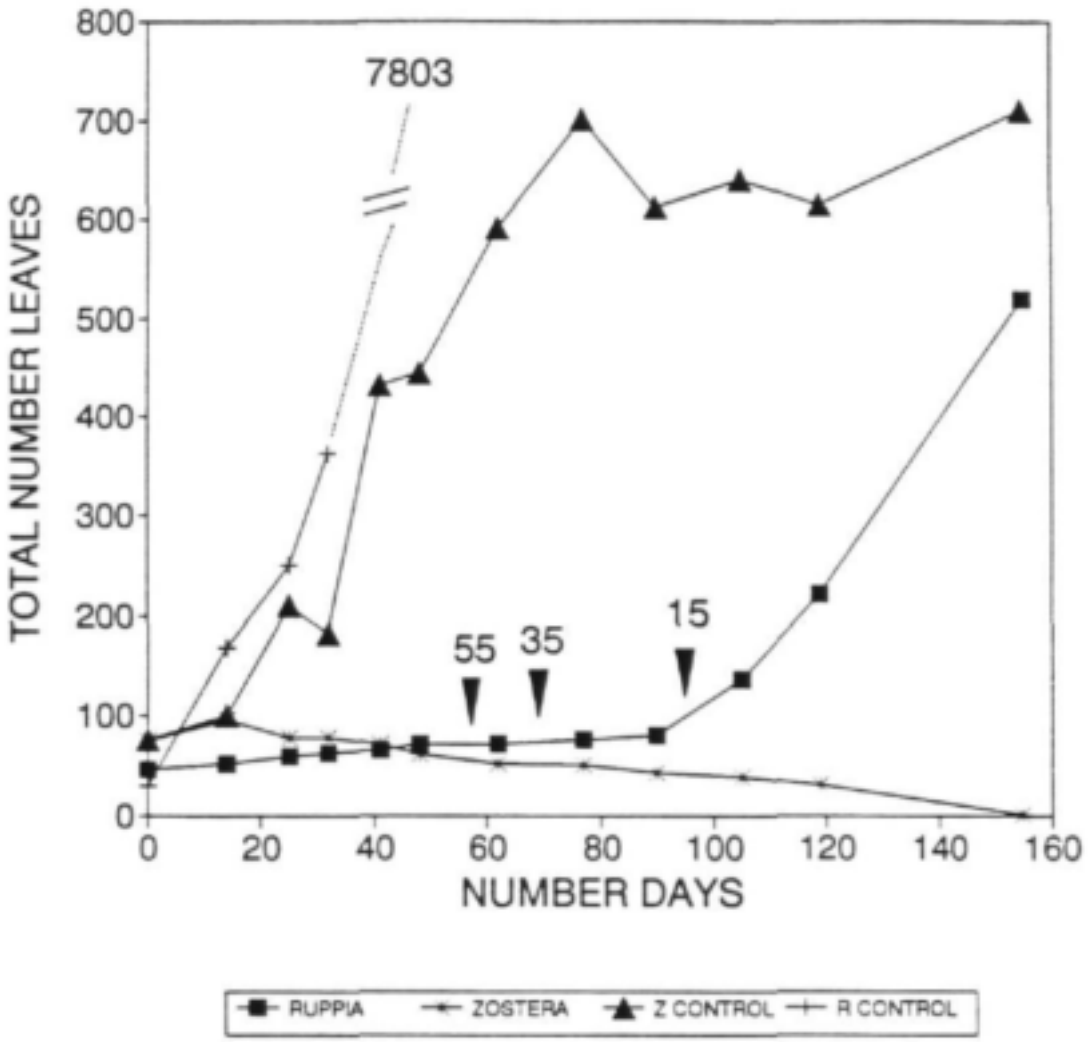


Figure 15 The recovery of *R. cirrhosa* and *Z. capensis* after high salinity treatment. Arrow heads indicate days salinity was reduced to 55, 35 and 15 ppt. The number of *R. cirrhosa* control leaves became too numerous to count and there were 7803 leaves when the tank was harvested.

Seed Germination

After 30 days, 32 %, 24 % and 14 % of *R. cirrhosa* seeds had germinated in salinity levels of 15, 0 and 35 ppt respectively. No germination occurred in 55 and 75 ppt treatments.

Discussion

These laboratory studies have shown that *Z. capensis* survived and grew best between 15 and 35 ppt. Field observations confirmed this as *Z. capensis* was dominant where the salinity was between 30-40 ppt but did occur between 15-25 ppt usually in association with *R. cirrhosa*. *Ruppia cirrhosa* was dominant in brackish estuaries (salinity < 30 ppt) and survived and grew best in freshwater in the laboratory. Under controlled laboratory conditions *R. cirrhosa* had a wider range of salinity tolerance than *Z. capensis*, as new growth was initiated at 55 and 75 ppt. This was not unexpected, since according to McMillan and Moseley (1967), *Ruppia* spp. under experimental conditions can withstand salinity levels up to 74 ppt.

The ability of *R. cirrhosa* to grow best in freshwater suggests the plant's possession of an effective salt tolerance mechanism rather than a requirement for salt. This ability to tolerate salt may be linked to the accumulation of proline as an osmoticum (Brock 1981a). Our study showed that for both *R. cirrhosa* and *Z. capensis*, leaf proline concentrations increased with an increase in treatment salinity. Proline is a compatible solute that maintains the osmotic balance of many plant tissues (Stewart and Lee 1974).

Ruppia cirrhosa has been observed to produce flowers over a wide salinity range (0 - 55 ppt). This is contrary to McMillan's observation (1974) that flowering of *Ruppia* spp. is confined to low salinity with seed set at a salinity of 28 ppt or less. Seed germination may have a different salinity optimum to that of vegetative growth (Tyerman 1989). Seeds may withstand high salinity levels and then germinate when the salinity is reduced (Rozema 1975 as cited in Vollebergh and Congdon 1986). A freshwater pulse into an estuary would be necessary to lower salinity and promote *R. cirrhosa* germination. Recolonization of hypersaline systems (55 ppt) could not occur by seedling establishment and growth as no germination occurred at 55 and 75 ppt.

Expansion growth of *R. cirrhosa* was greater than that of *Z. capensis* at salinity levels of 15 and 35 ppt when the plants were grown together in the same tank. This was true for the experimental conditions of reduced water velocities and constant temperature (20°C) and irradiance (600 μE m^{-2} s^{-1}). The plants co-existed at these salinity levels. This supports field observations from surveys of Cape estuaries where *R. cirrhosa* and *Z. capensis* were found to co-exist in calm sheltered environments. For example in the Klein Brak and Kleinriviervlei, *R.*

cirrhosa and *Z. capensis* coexisted at 15-16 ppt and in the Knysna estuary at 20-25 ppt. The location of the estuaries mentioned are shown in Figure 11. At higher salinity *Z. capensis* was dominant and was a better competitor than *R. cirrhosa*. According to Howard-Williams and Liptrot (1980), the horizontal zonation of *R. cirrhosa* in the Swartvlei estuary was maintained by competition with *Z. capensis* at the saline end (35 ppt) and *Potamogeton pectinatus* L. and Charophyta at the brackish (10 ppt) end.

The absence of *R. cirrhosa* from high salinity estuaries or the more marine end of estuaries, is possibly not related to salinity since our experimental studies have shown that it can survive over a wide range of salinity. The competitive advantage of *Z. capensis* over *R. cirrhosa* is, rather, related to the morphological differences between the two submerged angiosperms. In contrast to *Z. capensis*, *R. cirrhosa* has thin stems that lack supporting tissue and the rooting parts are poorly developed (Verhoeven 1979). *Ruppia cirrhosa* is, therefore, restricted to sheltered habitats i.e. periodically open estuaries and the upper reaches of open estuaries. *Zostera capensis*, because of its stronger morphological structure, has a competitive advantage over *R. cirrhosa* in estuaries where there is tidal exchange and stronger water currents. These are generally estuaries with open mouths, characterized by marine conditions.

The wide salinity tolerance range of *R. cirrhosa* makes it a more opportunistic species than *Z. capensis*. Growth is reduced at high salinity but it is not as sensitive as *Z. capensis* that will die after 1 month at a salinity of 75 ppt and 3 months at 55 ppt. In the event of a freshwater pulse, *R. cirrhosa* could regenerate from seeds and remaining shoots and rhizomes unlike *Z. capensis* that has a narrower salinity tolerance range. *Zostera capensis* can survive under freshwater conditions but biomass and bed density is reduced with the passage of time. Prolonged freshwater conditions in the St. Lucia estuary (28°23'S 32°26'E) reduced the density of *Z. capensis* beds (R. Taylor, pers. comm.).

Conclusion

Although this study did not directly test it, it is probable that *Z. capensis* outcompetes *R. cirrhosa* at a salinity of 35 ppt in estuaries where there is tidal exchange and strong water currents, because of its stronger morphological structure. On the other hand, *R. cirrhosa* has a competitive advantage over *Z. capensis* because of its broader tolerance to salinity. It can continue to grow in 55 ppt and higher and this may be due to the accumulation of proline. At a salinity less than 15 ppt, *R. cirrhosa* is a superior competitor to *Z. capensis* because of its ability to grow rapidly under these conditions. *Zostera capensis* is therefore dominant in estuaries with open mouths and in the lower marine end of estuaries, while *R. cirrhosa* is more common in estuaries that are only seasonally open or in the upper, calm, brackish reaches of estuaries.

3.1.2 THE TOLERANCE TO DESICCATION OF THE SUBMERGED MACROPHYTES *RUPPIA CIRRHOSA* (PETAGNA) GRANDE AND *ZOSTERA CAPENSIS* SETCHELL.

Introduction

Due to low seasonal runoff, most South African estuaries are naturally closed off from the sea for varying periods (Whitfield 1992). Freshwater impoundment in the catchment causes a further decrease in catchment run-off. This increases the period of mouth closure and, in temporarily closed estuaries, water levels drop, resulting in the prolonged exposure and die-back of macrophytes that are normally submerged. The present study was conducted to determine the tolerance to desiccation of *Ruppia cirrhosa* (Petagna) Grande and *Zostera capensis* Setchell.

Desiccation response of plants in the short term (hours) was measured using chlorophyll fluorescence (Fv/Fm ratio) as an indication of stress. Most of the light energy absorbed by green plants drives chemical reactions of photosynthesis. Excess energy is lost as heat, radiationless de-excitation and re-emission as light known as fluorescence (Anon. 1992). When light is first made available to dark-adapted plants, the fluorescence instantaneously reaches its initial level (Fo) and after about 0.15 s it rises to a maximum value, Fm. Variable fluorescence, Fv is equal to Fm-Fo (Blanco *et al.* 1992). The ratio Fv/Fm gives an estimate of photosynthetic capacity, i.e., the efficiency of energy capture by open Photosystem II (PS II) centres (Bolhàr-Nordenkampf and Öquist 1993). PS II is particularly sensitive to most stress factors including dehydration (Bjorkman and Demmig 1987). The theory of chlorophyll fluorescence emission and instrumentation has been reviewed by Bolhàr-Nordenkampf *et al.* (1989).

Besides the fluorescence studies, desiccation tolerance and recovery of submerged plants after exposure was also studied on a weekly basis. The effect of repeated desiccations on the plants was investigated by exposing plants for 5h daily and monitoring leaf production over a 6 week period. This was studied to determine whether the differences in desiccation tolerance between the two plants could explain the absence of *R. cirrhosa* from intertidal habitats in South Africa estuaries. In a separate series of experiments the rate of desiccation was measured under field conditions.

Methods

Short term desiccation.

Fluorescence measurements are now widely used in studies on the effects of environmental stress (Bjorkman and Demmig 1987). In this study the early detection of reduced photosynthetic capacity due to desiccation stress was achieved non-destructively by measurement of chlorophyll fluorescence using a portable fluorimeter, the Hansatech Plant Efficiency Analyzer (Hansatech Instruments Ltd.). The Hansatech Plant Efficiency Analyzer (PEA) was operated according to manufacturers instructions (Anon 1992).

Initial fluorescence readings (Fv/Fm) were made on whole wet plants (weighing approximately 0.5 g) that had been growing in aerated tanks. The plants were removed from the tanks and placed in petri dishes filled with water so that the leaves and attached leaf clips remained submerged during the readings. In order to determine the effect of desiccation on the plant three replicate plants per treatment were then exposed on glass petri dishes for varying periods, i.e. 30, 60, 90 and 120 min., in a growth cabinet at 25°C. Fluorescence measurements before and after the desiccation period were compared. Recovery was tested by returning the plants to their aerated tanks and measuring fluorescence after 2, 4, 24 and 96 h of submergence. The control consisted of plants that remained submerged in their tanks. Although this may not simulate *in situ* conditions for *Zostera capensis*, which can be exposed for up to 4h per day, previous experimental work has shown that it flourishes under completely submerged conditions.

The statistical package SOLO (BMDP Statistical Software Inc. 1988) was used for one-way analyses of variance comparing Fv/Fm ratios before and after desiccation and recovery from desiccation. When significant F-values were detected, means were separated by the Newman-Keuls multiple range test.

Rate of desiccation

Plants were desiccated under natural light (~2000 μmol m^{-2} s^{-1}), temperature (20°C) and wind to simulate field conditions. Plant portions weighing approximately 0.5 g were placed on glass petri dishes. Six replicate plants were used. After every 15 min. for a 90 min. period the plants were weighed to determine the different levels of desiccation. Levels were expressed as % desiccation (Hodgson 1981), where:

$$\% \text{ Desiccation} = \frac{\text{initial wet mass - final wet mass}}{\text{initial wet mass - dry mass}} * 100$$

Plants were dried overnight at 70°C to obtain the dry mass. The initial wet mass represents 0 % desiccation and the dry mass 100 % desiccation.

Long-term desiccation

This experiment was designed to determine whether partly desiccated submerged macrophytes can recover from exposure. The two species were placed in separate tanks and after a two-week acclimatization period, the water was removed from the tanks. The tanks were placed in a controlled environmental cabinet with the temperature set to a constant 20°C. Fluorescent and incandescent lights were set on a 12:12 h light/dark cycle and provided ~ 500 μmol $m^{-2}.s^{-1}$.

After 1, 2 and 3 weeks exposure, plants (3 in each case) were removed and resubmerged. An estimate of recovery was determined by measuring the number of leaves on the resubmerged plants for 5 weeks. To examine the effect of repeated desiccations, *Ruppia* and *Zostera* plants were exposed for 5h each day for 6 weeks. This simulates the natural condition for *Zostera* but not for *Ruppia* that is normally found only in submerged habitats. The controls were completely submerged plants of both species. Measurements were made each week of the number of leaves on each of 10 replicate plants.

Results

Short term desiccation

For both *Zostera* and *Ruppia* plants, the Fv/Fm ratios prior to the treatment were significantly higher ($F = 10.3$, $P < 0.001$) than after exposure (Fig. 16). In the case of *R. cirrhosa*, as the degree of desiccation (exposure time) increased, the Fv/Fm ratios decreased significantly ($F = 110.5$, $P < 0.001$, Fig. 16A). A Newman-Keul's range test showed that the Fv/Fm ratio after 30 min. exposure was significantly higher ($P < 0.05$) than after longer exposure periods. The Fv/Fm ratios after 60 and 90 min. exposure did not differ significantly ($P > 0.05$) but were, in turn, significantly higher than the ratio for plants exposed for 120 min. (Fig. 16A).

For *Zostera capensis* the Fv/Fm ratio had decreased sharply after 30 min. exposure. There was no significant decrease ($P > 0.05$) in the ratio after that (Fig. 16B), i.e. the Fv/Fm ratio had reached a constant minimum level after 30 min. of exposure.

Exposed plants were resubmerged in water and the Fv/Fm ratio measured after 2, 4, 24 and 96h. There was no recovery after either 2 or 4h. The Fv/Fm ratio was < 0.1 for both species (Fig. 17). After 24h of recovery the Fv/Fm ratio for *Ruppia* plants exposed for 30 min. was significantly higher ($F = 5.15$, $P < 0.01$) than for plants experiencing longer exposure periods (Fig. 17A). Therefore, plants exposed for short periods recovered more rapidly than plants exposed for longer periods.

Zostera capensis recovered better than did *R. cirrhosa* after 24h of re-submergence. The Fv/Fm ratios after 24h were significantly higher ($F = 128.7$, $P < 0.0001$) for *Z. capensis* than for *R. cirrhosa* (Fig. 17). However, the Fv/Fm ratios for *Z. capensis* after 24h recovery were still lower than the initial values had been. After 96 h both *Z. capensis* and *R. cirrhosa* showed complete recovery. The Fv/Fm ratio increased to the initial value for all desiccation treatments (Fig. 17). This was due to new basal leaf growth. The desiccated leaf portions remained chlorotic and brown.

Rate of desiccation

Experiments using plant segments out of their rooting medium and conducted under natural light and wind conditions showed that most desiccation occurs in the first 30 min. after exposure. *Zostera capensis* was more severely desiccated than was *R. cirrhosa* (Fig. 18). These data are necessary when comparing the Fv/Fm ratios for the two species (Fig. 16). This aspect is discussed later.

Long term desiccation

Ruppia cirrhosa and *Z. capensis* plants exposed for 1 week did not recover when resubmerged. Brown desiccated leaves were abscised on a daily basis and by the fifth week of resubmergence, both species had lost all their leaves. The rate at which leaves were lost was unaffected by the desiccation treatment.

In the experiments which tested repeated partial desiccation by exposing plants for 5h daily over a period of 6 weeks, *Ruppia cirrhosa* was found to be very sensitive. The average number of leaves left on plants exposed for 5h daily were significantly lower than on control plants ($F = 10$, $P < 0.05$, Fig. 19). By the fourth week all the leaves were brown and by the fifth week all had been abscised. There was no significant difference between average number of leaves on the control and the treatment plants for *Z. capensis* ($F = 1.77$, $P > 0.05$).

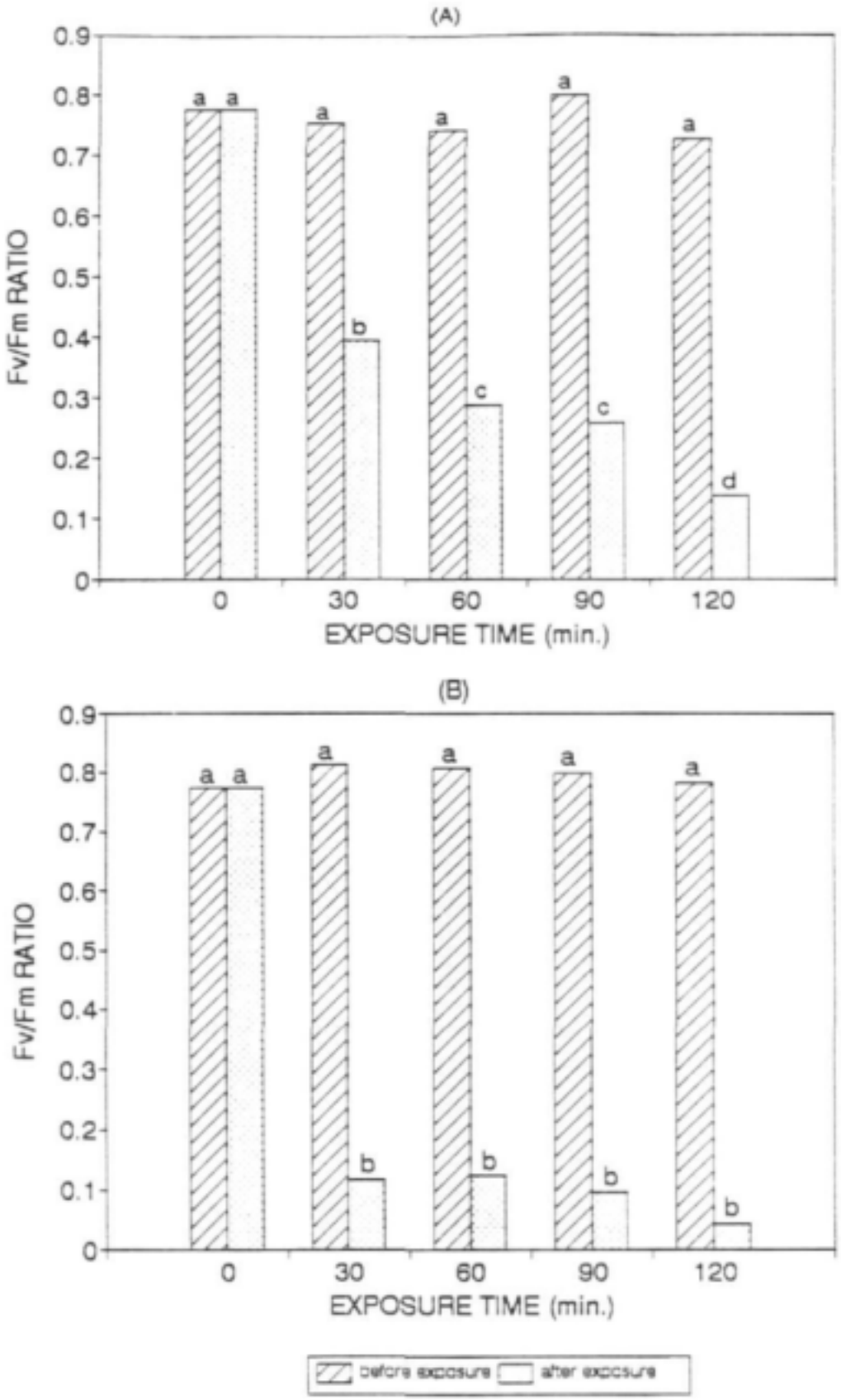


Figure 16 Fv/Fm ratios for *Ruppia cirrhosa* (A) and *Zostera capensis* (B) before exposure and after different exposure periods. Means with different letters (a-d) were significantly different at $P \leq 0.05$ using the Newman-Keuls multiple range test.

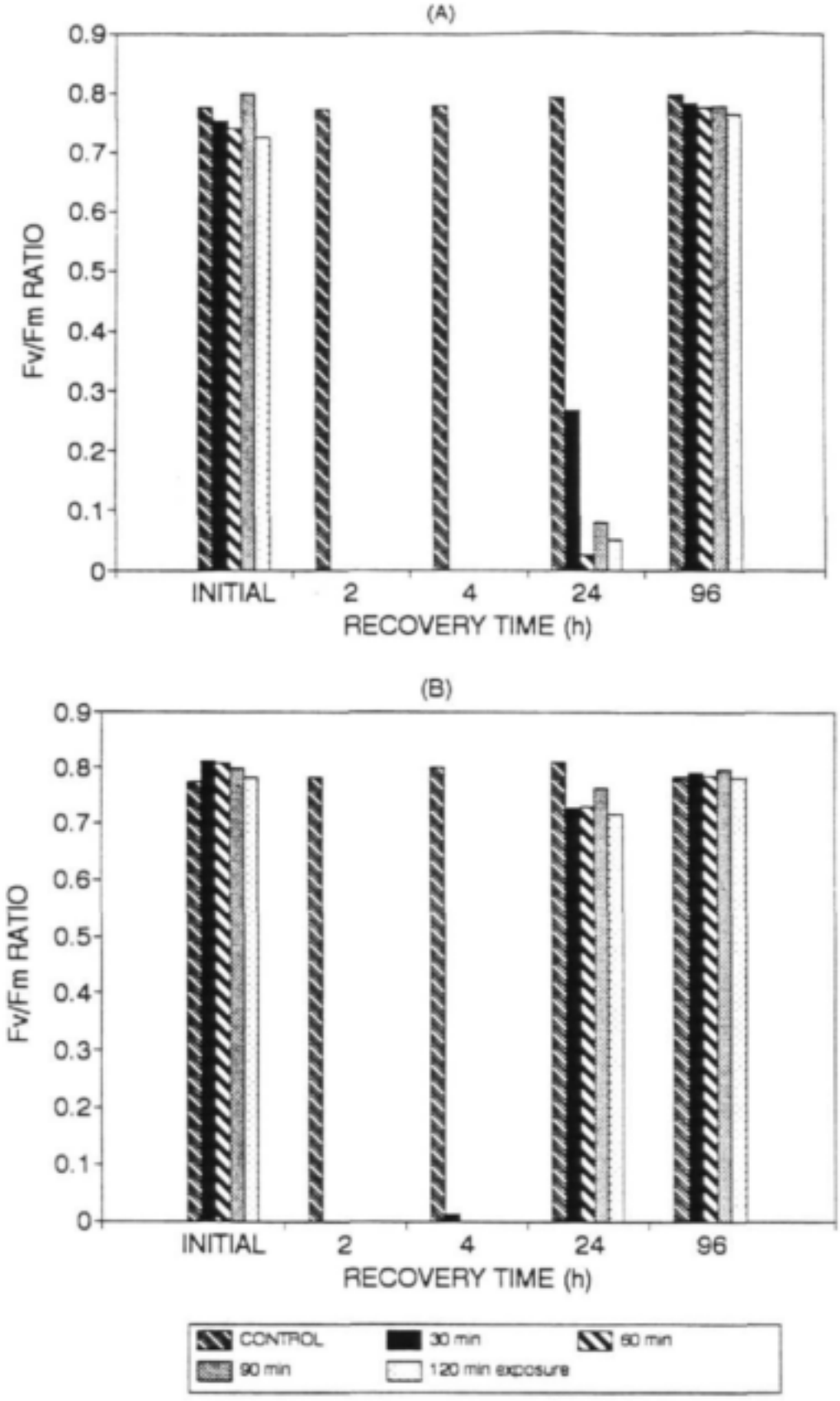


Figure 17 Fv/Fm ratios after 2 - 96 h recovery for *Ruppia cirrhosa* (A) and *Zostera capensis* (B) for different desiccation treatments (n = 6).

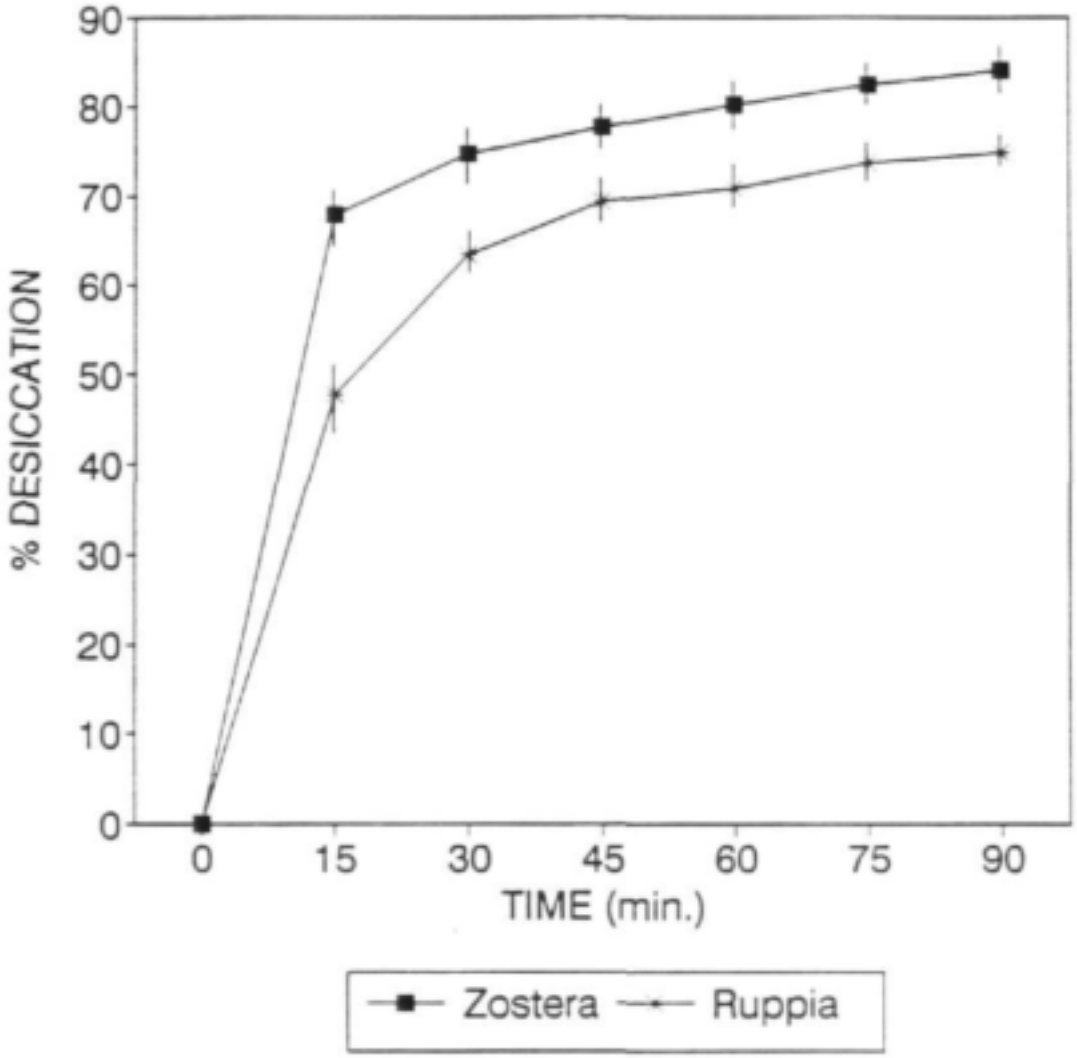


Figure 18 Rate of desiccation of *Ruppia cirrhosa* and *Zostera capensis* under field conditions (Bars = standard error, n = 6).

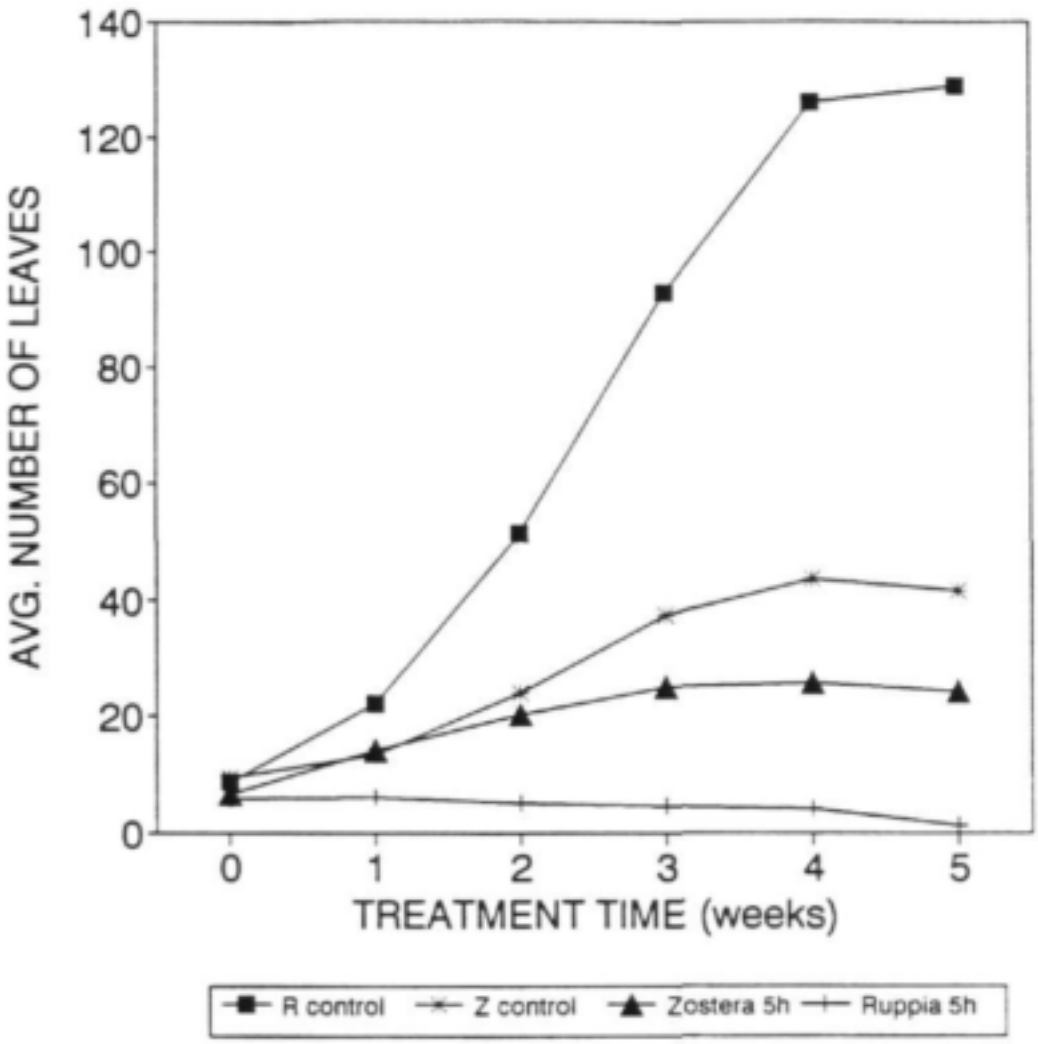


Figure 19 Change in average number of leaves (n = 6) for *Ruppia cirrhosa* and *Zostera capensis* exposed for 5h daily. (R control = *Ruppia* submerged, Z control = *Zostera* submerged).

Discussion

Many authors have shown that the ability of a desiccated seaweed to recover after re-immersion is a good indication of its adaptation to the intertidal zone (Ogata and Matsui 1968, Dawes and Kovach 1992). Intertidal macroalgae that grow high on the shore can survive due to photosynthetic recovery after desiccation (Wiltens *et al*. 1978, Dring and Brown 1982, Brown 1987). This study showed that submerged macrophytes such as *Ruppia* and *Zostera*, that do not show a recovery in fluorescence after 2-4h, will do so after 24h of resubmergence. This recovery is related to the rapid growth of new leaves and not the recuperation of desiccated leaves. The desiccated leaves turned brown and were abscised. In some cases new leaf growth was observed at the base of the desiccated leaf.

Zostera capensis recovered more rapidly after exposure than did *R. cirrhosa*. *Ruppia cirrhosa* plants exposed for 2h took 4 days to recover, whereas *Z. capensis* plants exposed for the same time, recovered after 1 day. *Zostera capensis* has a leaf sheath which protects the basal meristems. In the field, seagrass leaves are frequently scorched during spring low tides, however recovery of leaf growth by means of the basal meristems is rapid (Gessner 1971, Clarke and Kirkman 1989).

Under natural light and wind conditions, *Z. capensis* dried out more rapidly than did *R. cirrhosa*. This corresponds with the short term fluorescence experiments where the Fv/Fm ratios of *Z. capensis* decreased rapidly after 30 min. exposure. *Ruppia cirrhosa* plants became increasingly stressed as the period of desiccation increased from 30 to 120 min.

Zostera capensis was 75 % desiccated after 30 min. exposure whereas *R. cirrhosa* was only 63 % desiccated (Fig. 18). Gessner (1971) found that the tolerance limit fo. water loss in adult *Thalassia testudinum* leaves was about 65 %. Seagrasses appear to have no physiological barrier to water loss. *Thalassia testudinum* leaves have a thin cuticle but it is multiperforated. The cuticle is therefore not as important as overlapping leaves in providing the plant with a high degree of desiccation tolerance (Gessner 1971). The same applies to *Z. capensis*, which according to Barnabas (1979) has a thin cuticle attenuated in places and pierced by minute, valve-like openings. Overlapping leaves would protect young leaves and intercalary meristems from desiccation.

The submerged macrophytes *Z. capensis* and *R. cirrhosa* recovered from short exposure periods (i.e. 120 min.), but neither species recovered from a long (1-3 week) period of desiccation. Once the plants were resubmerged, the brown leaves simply abscised and no

new leaf or rhizome growth occurred. The preliminary rules used in the expert system stated that "after a 1 month exposure period submerged macrophytes will die-back". This laboratory study has shown that plants did not recover after an exposure of 1 week. These data, however, were produced under constant environmental conditions.

Under field conditions, the rate of desiccation will depend on ambient light, temperature, wind and the nature and topography of the substrate. Epiphytic cover may also contribute to desiccation tolerance. Talbot and Bate (1987) found that waterlogged conditions of creek sediments allowed *Zostera* plants to meet the evaporative demand of their large leaves during tidal emergence. Water may be retained in small pools or trapped within the seagrass beds during low tides (Plate 3). For example a meadow of *Thalassia testudinum* retained a thin layer (< 20 cm) of water for 8h during low tides, preventing desiccation of the bank (Powells and Schaffner 1991).

Zostera capensis is adapted to its tidal habitat. There was no significant difference between leaf production for submerged plants and those exposed for 5h daily. A 5h daily exposure was lethal for *R. cirrhosa*. *Zostera capensis* can withstand repeated cycles of drying during daily low tides and can therefore possibly outcompete *R. cirrhosa* in tidal habitats.

In South African estuaries, *Ruppia cirrhosa* is common in estuaries that are only open periodically. The characteristic environment in such areas is fluctuating salinity and water levels (Adams *et al*. 1992). This species can also be found in upper, calm brackish reaches of estuaries. *Zostera capensis* is dominant in the middle and lower marine reaches of permanently open estuaries. This study has shown that *Z. capensis* may have a competitive advantage over *R. cirrhosa* in the middle and lower reaches of tidal estuaries as it is adapted to survive periods of exposure.

Zostera capensis also outcompetes *R. cirrhosa* in estuaries where there is tidal exchange and strong water currents, because of its stronger morphological structure. According to Sand-Jensen and Borum (1991), rooted macrophytes with basal growth, protected meristems, strong root systems and flexible leaves (eg. *Z. capensis*) typically dominate under conditions of high exposure. Rooted macrophytes with apical growth, extensive ramification and long slender stems on the other hand, often dominate under more sheltered conditions (eg. *R. cirrhosa*).

Studies in which two local periodically open estuaries, the Seekoei and Kabeljous (Fig. 11), were monitored, have shown that, over a period of 1 month, there was complete die-back of *Ruppia cirrhosa* following a drop in water level that exposed the beds (Plate 4). The resistance

of the vegetative parts to drying is very low and, after exposure, all plant parts except ripe seeds die within a few days (Verhoeven 1979). The plant is adapted to an ephemeral habitat due to its large production of seeds. After localized rainfall events, water levels rise and the plants grow rapidly from a persistent seed bank. Van Vierssen *et al.* (1984) found that the viability of *Ruppia maritima* seeds was hardly affected by 2-10 weeks desiccation. Analysis of our seed banks have shown that *Ruppia* can have as many as 4850 seeds m^{-2}.

Brock (1981b, 1982) has described the life-cycle strategies of three *Ruppia* spp. They are the perennial *R. megacarpa*, which is not adapted to survive ephemeral habitats and the annuals, *R. tuberosa* and *R. polycarpa* that produce many seeds and perennating organs (turions) which survive harsh and desiccating conditions. Rewetting stimulates the growth of the annual *Ruppia* and turions could regenerate more than once after wetting, drying and subsequent rewetting. *Ruppia cirrhosa* found in periodically open South African estuaries appears to have an annual life-cycle. Desiccation and regrowth of the plants is not strictly controlled by the seasons but by periods of drought and low water levels.

Conclusion

Ruppia cirrhosa is absent from marine intertidal habitats where *Z. capensis* is common. This appears to be related to differences in desiccation tolerance. *Zostera capensis* plants exposed for 2h took one day to recover, whereas *R. cirrhosa* plants took four days. Daily exposure of 5h caused complete die-back of *R. cirrhosa* within five weeks, but *Z. capensis* survived these repeated drying cycles. *Zostera capensis* has a leaf sheath that protects the basal meristem and new leaves grow rapidly from this area within a few days. Neither plant recovered from an exposure of one week. In the field, the rate of desiccation will depend on ambient conditions and sediment water-holding capacity. *Zostera capensis* is dominant in tidal marine estuaries because of its stronger morphological structure and ability to survive daily periods of exposure. *Ruppia cirrhosa* is more sensitive to desiccation, but is adapted to ephemeral habitats that dry out periodically, because of its large seed production. The seeds can also survive long periods of desiccation.

Plate 3 Water is retained in the *Zostera capensis* beds during low tide.

Plate 4 During periods of reduced freshwater input, water levels drop, *Ruppia cirrhosa* beds are exposed and die-back.

3.2 SALT MARSH MACROPHYTES

3.2.1 THE EFFECT OF SALINITY AND INUNDATION ON *SARCOCORNIA PERENNIS* (MILL.) A.J. SCOTT

Introduction

Information on the ecophysiological responses of estuarine plants is important as it allows predictions about plant growth and survival under different water management scenarios. High salinity levels (> 45 ppt) have been observed to cause impoverishment of the estuarine flora (Adams *et al*. 1992). In addition to salinity stress, estuarine plants are sometimes subjected to waterlogging and inundation stresses. In periodically closed estuaries, during closed mouth conditions water levels rise due to localized freshwater run-off and this results in the inundation of salt marshes for extended periods. For this reason, management decisions have to be made about the length of time intertidal plants can survive under prolonged submerged conditions before the mouth of the estuary needs to be mechanically opened.

Sarcocornia perennis (Mill.) A.J. Scott (Chenopodiaceae) was chosen as a representative intertidal salt marsh macrophyte as it occurs in a number of South African estuaries. It is characterized by succulent, articulated and apparently leafless stems (Plate 5) and can form mats up to 25 cm high, the main branches are prostrate and rooting often occurs at the nodes (Tölken 1967).

Sarcocornia is adapted to a wide range of environmental conditions as it is naturally subjected to both flooding by freshwater in the rainy season and is often inundated by tidal seawater (Tölken 1967). The effect of a wide range of salinity and inundation conditions on *S. perennis* growth was, therefore, studied.

Methods

Adult *Sarcocornia perennis* plants were collected in August 1992 from the Gamtoos estuary (33°58'S, 25°04'E) and acclimatised in a glasshouse for one week. Apical vegetative segments were removed and rooted in a mixture of estuarine mud and sand. Once the segments had grown to approximately 10 cm, uniform plants were selected and planted five to a pot. Temperatures in the glasshouse ranged from 18 to 34°C. The windows of the glasshouse roof are painted with white paint and at midday photosynthetic photon flux density (PPFD) ranged from 1740 to 1800 μE m^{-2} s^{-1}.

The experiment was a 5 x 3 (salinity x inundation) factorial design with 3 replications (pots) per treatment. Each pot contained 5 plants. The salinity treatments were 0, 15, 35, 55 and 75 ppt. and inundation treatments: I1 - substrate damp i.e. free drainage but not allowed to become dry; I2 - substrate saturated and I3 - water level above the plants (completely submerged). Saturation (I2) was achieved by placing the perforated pots in plastic trays filled with the relevant salinity solution. For the I3 treatments the plant pots were placed in larger undrained pots filled with relevant salinity solution. In order to minimize the effects of any potential variation in environmental conditions (e.g. patchy illumination), a randomized block design was employed.

Plants were watered at least twice weekly in order to maintain the treatment levels of salinity and inundation. Measurements were made each week of stem elongation and the number of side branches produced. After 6 weeks the plants were harvested. Data were subjected to analyses of variance using the SOLO statistical package (BMDP Statistical Software, Inc. 1988). When significant F-values were detected, means were separated by the Student Newman-Keuls multiple range test. All differences reported are significant at $P \leq 0.05$ unless otherwise indicated.

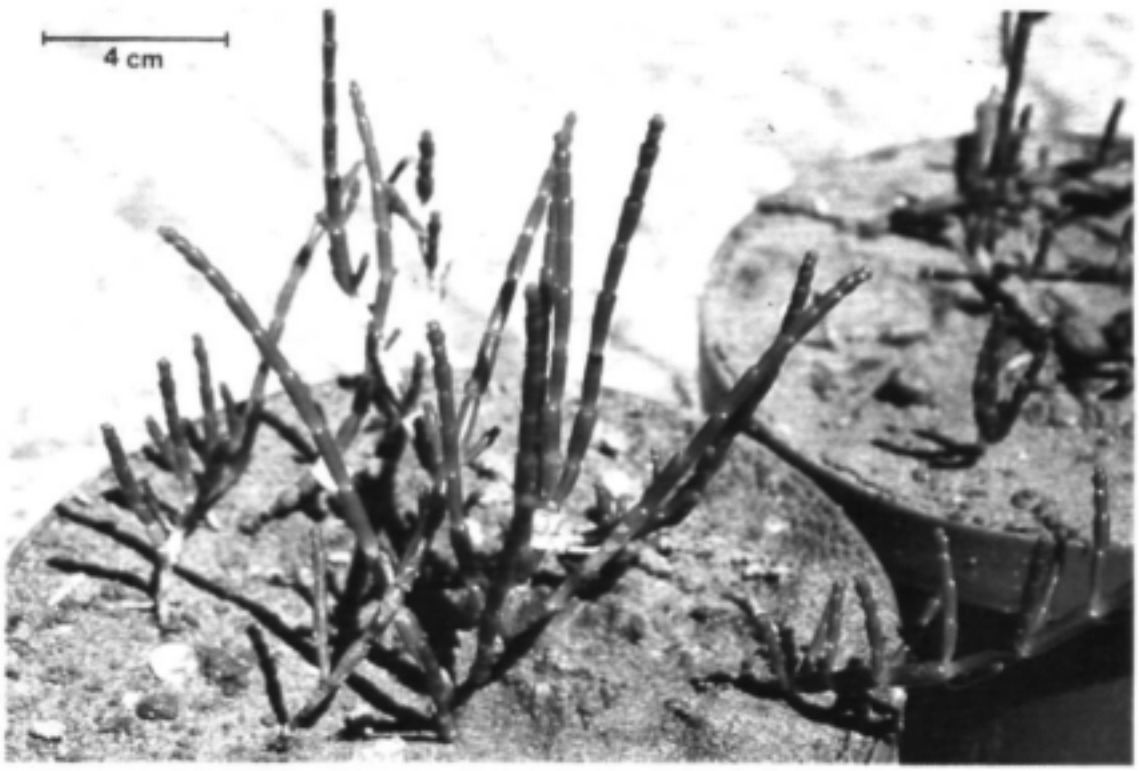


Plate 5 *Sarcocornia perennis* - note the succulent, fused leaves.

Results

Analysis of variance indicated that weekly stem elongation ($F = 9.14$, $P < 0.0001$) and branch production ($F = 9.62$, $P < 0.0001$) were significantly affected by the salinity and inundation treatments. Mean stem elongation data for the 6 week period (Fig. 20) indicated that plants grew best in salinity less than 35 ppt and in substrates saturated with freshwater. Plants treated with 55 and 75 ppt showed signs of stress after 2 weeks (i.e. plants lost turgor and wilted). Growth was reduced in the damp treatments for all salinity ranges (Fig. 20).
At salinity levels of 35 ppt and higher, submerged plants grew better than in the other inundation treatments (Fig. 20). This can be attributed to the elongation of nodes and the extension of stems to a position above the water surface. Plants with their stems approximately 5 cm below the water surface showed this elongation but, smaller more deeply submerged plants, at the same salinity, did not grow significantly.

The largest number of side branches were produced by plants in the saturated treatments, indicating that the plants are adapted to *in situ* saturated substrates maintained by regular tidal inundation (Fig. 21). Completely submerged conditions caused branch production to be reduced. For the damp and saturated treatments, branch production decreased under high salinity. Branch production was especially low at 55 and 75 ppt (Fig. 21).

The highest mortality occurred in the treatment where plants were completely submerged (Table 4), at low salinities of 0 ppt (7 plants) and 15 ppt (3 plants). The succulent leaves of *Sarcocornia perennis* decomposed more rapidly at low salinity. In salinity levels less than 35 ppt submerged *S. perennis* plants decomposed and in salinity levels greater than 35 ppt the plants were yellow indicating necrosis and senescence. Although the leaves decomposed, the stem which runs along the entire length of the succulent section remained alive. After 4 weeks, new leaves began to form on the stem. The mortality data (Table 4) indicate that plants are more sensitive to submerged conditions than they are to high salinity.

Table 4 The mortality (number of plants which were dead) of *Sarcocornia perennis* individuals at harvest. Each value is based on an initial 15 plants per treatment.

	INUNDATION TREATMENT			
SALINITY (ppt)	I1	I2	I3	TOTAL
0	4	0	7	11
15	0	1	3	4
35	1	0	0	1
55	3	4	1	8
75	1	2	0	3
TOTAL	9	7	11	

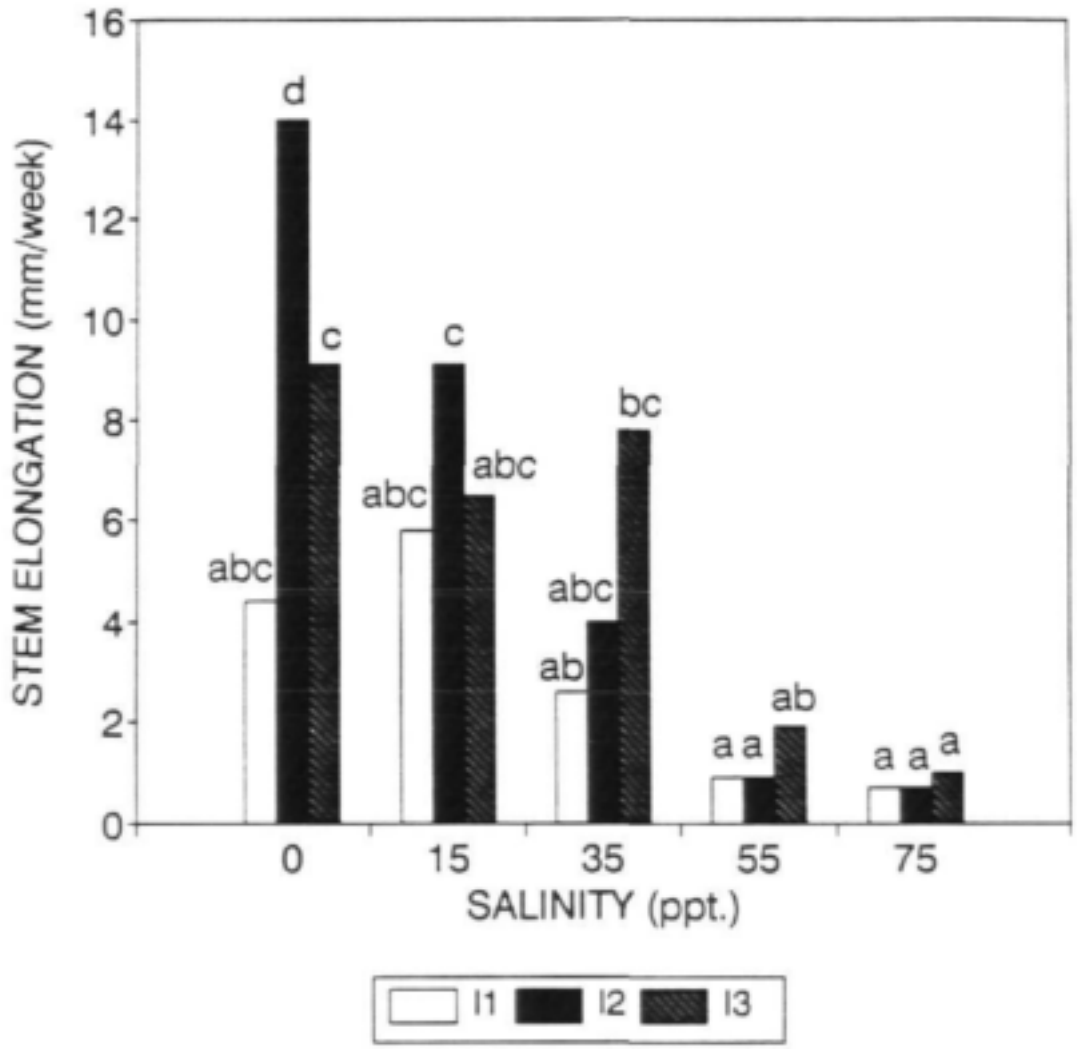


Figure 20 The effect of salinity and inundation on *Sarcocornia perennis* stem elongation. Means with different letters (a-d) were significantly different at $P \leq 0.05$ using the Student Newman-Keuls multiple range test. Treatments: I1 = damp, I2 = saturated and I3 = submerged.

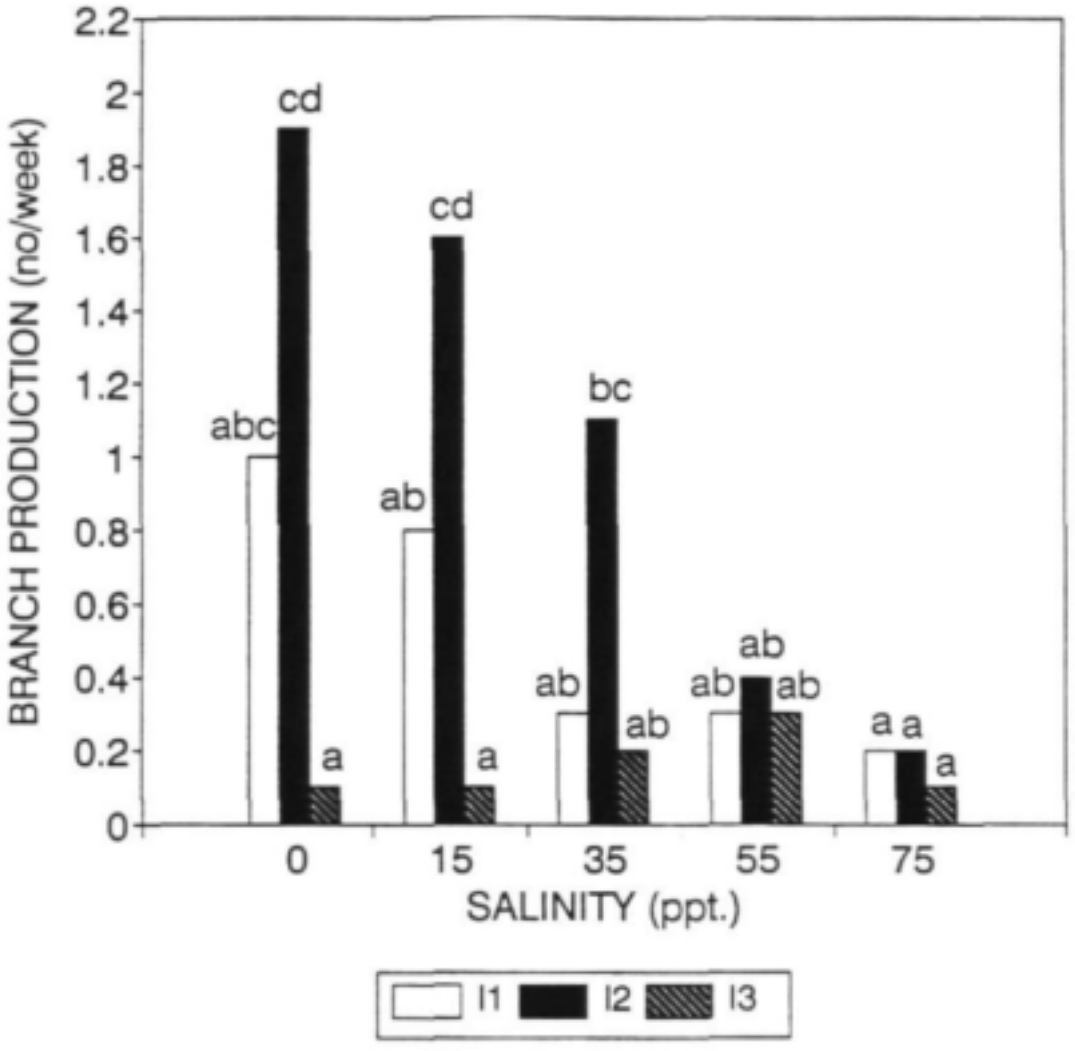


Figure 21 The effect of salinity and inundation on *Sarcocornia perennis* branch production. Abbreviations as in Fig. 20.

Discussion

This study has shown that salinity levels higher than 35 ppt and submerged conditions can cause a reduction in the growth of *S. perennis*. Plants began to show signs of stress after two weeks. Plant mortality data (Table 4) indicated that plants survived conditions of high salinity better than they did submerged conditions. These results compared well with Tölken's observation (1967) that *S. natalensis* (Bunge ex Ung.-Sternb.) A.J. Scott could endure submergence for up to 3 months, but *S. perennis* could only endure short periods of inundation.

Monitoring of permanent quadrats in the Seekoei and Kabeljous estuaries (Fig. 1), have shown that when water levels drop, the submerged plant *Ruppia cirrhosa* (Petagna) Grande dies back and is replaced by *Sarcocornia perennis*. The latter plants germinate from a large seed bank (12 284 seeds m^{-2}; Dobkins 1990). After rainfall events, water levels rise and the *Sarcocornia* community is inundated. Plants that are completely submerged rot and decompose rapidly. This laboratory study showed that succulent leaves of *S. perennis* decomposed more rapidly at low salinity presumably because of an osmotic potential gradient driving water into the leaves causing them to swell and rupture. Subsequent bacterial activity would also be higher in freshwater thus aiding rapid decomposition.

Sarcocornia perennis plants submerged initially at approximately 5 cm below the surface, showed rapid stem elongation. Promotion of extension growth includes elongation of stems or petioles and is a response of aquatic plants or marsh dwellers to submergence (Jackson and Drew 1984), but has not previously been reported for *Sarcocornia* spp. The hormone ethylene is thought to be responsible for this "depth accommodation" (Ridge 1987, Jackson 1990) which ensures that a proportion of the foliage is raised above the water. Although, in this study, stem elongation was an effective mechanism for overcoming the negative effects of submergence, the overall growth and production of submerged branches of *S. perennis* plants was reduced (Fig. 12).

Best growth was recorded for freshwater, saturated soil treatments indicating that *S. perennis* does not necessarily have a physiological requirement for salt. Clarke and Hannon (1970) found that *Arthrocnemum australasicum* (Moq.) Moss had a small but positive salt requirement, while other laboratory experiments have shown that halophytes show increased productivity at lower salinity (Barbour and Davis 1970, Parrondo *et al.* 1978).

Sarcocornia perennis growth was reduced at 55 and 75 ppt. The excess soluble salts serve to increase the osmotic potential of the sediment thus inhibiting adequate water uptake (Flowers *et al*. 1986). The plants, therefore, lose water to the environment and wilt. From surveys of a number of South African estuaries, *S. perennis* was found in salinity ranging from 12 ppt to 42 ppt. According to O'Callaghan (1992), *S. perennis* occurred in the intertidal zone of the Langebaan lagoon (33°03'S 17°58'E) where salinity varies from 15 to 140 ppt. From these data, plants will probably survive fluctuating conditions of salinity higher than 35 ppt. This laboratory study provided near-constant conditions of salinity and waterlogging rather than the fluctuating conditions that commonly occur in the field.

The impact of changes in water level on marsh plants such as *S. perennis* will depend on the duration, intensity and frequency of the event, as well on the phenology of the plant at the time (Ernst 1990). Many South African estuaries are seasonally closed. Dam construction in the catchment has increased the frequency and duration of mouth closure (Whitfield and Bruton 1989). If an estuary mouth is closed and localized freshwater run-off is high, salt marshes can become inundated for extended periods. Inundation during the growing season is generally harmful to most marsh species (Olff *et al*. 1988). *Sarcocornia perennis* is a perennial marsh plant but vegetative and reproductive growth is greatest in spring. **A management recommendation is, therefore, that during spring, plants should not be submerged or exposed to salinity greater than 35 ppt.**

Sarcocornia perennis flowers from January to June. Wind and rain aid the release of seeds and if the soil is moist enough, the seeds germinate within 2 days and the seedlings establish themselves within a week (Tölken 1967). Seeds and seedlings are more salt sensitive than are established plants (Broome *et al*. 1988) and, therefore, during this period, a salinity of less than 35 ppt would ensure plant survival. It is not known whether *S. perennis* would germinate or whether seedlings would survive if they were submerged.

If *S. perennis* communities are flooded for prolonged periods plants will not flower (O'Callaghan 1992) and produce seed. Although propagation occurs primarily by vegetative reproduction in established salt marshes, in bare areas seedling establishment from a resident seed bank may be more important and, therefore, prolonged flooded conditions should be avoided if possible.

Conclusion

Salinity greater than 35 ppt and completely submerged conditions reduced *S. perennis* growth. The highest mortality of S. perennis plants occurred with the submerged treatments. Plant grew best in saturated treatments (0 and 15 ppt) compared to drained treatments. Regular tidal inundation of the *Sarcocornia* marsh is therefore important. Submerged S. perennis plants that occurred 5 cm below the water surface showed rapid stem elongation above the water surface. The succulent leaves below the water surface decomposed and this was more rapid in low salinity treatments. Inundated conditions also prevent flowering and seed production thus, reducing the potential for propagation from seed once water levels subside.

3.2.2 THE EFFECT OF SALINITY AND INUNDATION ON *SPARTINA MARITIMA* (CURTIS) FERNALD.

Introduction

Spartina maritima forms extensive monotypic stands in estuaries along the south coast of South Africa. According to Pierce (1982), *S. maritima* may be an exotic that increases sediment stability in these estuaries and encroaches into the *Zostera capensis* zone. Whether or not *Spartina* is an exotic, it has become an important primary producer in many of our estuaries. Autochthonous carbon supplied by marsh plants such as *Spartina* are important in South African estuaries during periods of low freshwater inflow (Taylor 1987). The marsh area also forms an extensive habitat for typical estuarine faunal species eg. the marsh crab, *Sesarma catenata*.

To understand and manage an estuary, a knowledge of the basic ecophysiological tolerances of the plants are important. Extensive studies overseas have been conducted on the various *Spartina* species (i.e., *S. alterniflora*, *S. patens*, *S. foliosa*, *S. cynosuroides*, *S. anglica)*. These studies have dealt with a wide range of topics eg. nutrition, salinity, inundation responses, primary production, marsh creation and genetic variation. Two of the most important factors affecting plant growth and survival are salinity and waterlogging. However the effect of these two stressors on plant growth and survival are poorly understood (Naidoo *et al*. 1992).

This study aimed to document the effect of a wide range of salinity (0 - 75 ppt) on the growth of *S. maritima*. The response of *S. maritima* to three inundation treatments was also examined. The first treatment simulated dry conditions with a simultaneous increase in salinity. This is a real situation in South African estuaries as mouth dimensions are shrinking due to reduced freshwater inflow which increases marine sediment accumulation. The estuary tidal prism is reduced, thus reducing tidal exchange and flushing of marshes. The second inundation treatment simulated normal *Spartina* marsh conditions, i.e., diurnal tidal fluctuations, while the third treatment was stagnant with completely submerged conditions.

Methods

Plants were collected in the Swartkops estuary from stands that were uniform in size and density of stems (Kirkman and Sharitz 1993). The plants were collected with an associated 25 x 30 cm soil column and transferred to pots of similar dimensions and kept inside a glasshouse. The plants were allowed an acclimation period (one week prior to the initiation of the experiment), during which salinity levels were gradually increased by step increments.

The experiment began on the 18/7/93 and the plants were harvested after 3 months. The approximate range of temperatures in the glasshouse was between 18 - 38°C. The plants were exposed to 90 % full sunlight because the glass roof was painted white. A 5 x 3 factorial experiment was established, with five salinity levels (0, 15, 35, 55 and 75 ppt) and three inundation treatments:

I1 - watered three times a week, free drainage,

I2 - tidally inundated, with 6h between high and low tides,

I3 - completely submerged.

Four pots were used for each treatment and in each pot five replicate plants were monitored. For the I1 treatment, plant pots were placed in plastic trays and watered three times a week. This simulated dry marsh conditions. In the I2 treatment, plant pots were placed in larger containers. Pumps and timers were set up so that the plants were subjected to a tidal regime with 6h between high and low tides. Completely submerged conditions were achieved by placing the plant pots in larger containers and maintaining the water level at 60 cm above the sediment surface.

Growth rate was monitored in the glasshouse experiments by measuring stem and leaf elongation and stem density at weekly intervals. Stem elongation was determined by measuring stem height of five plants in each replicate pot. Data presented are mean stem elongation, leaf elongation and stem production rates over time. Every second week a sediment sample was collected from each pot for salinity determination. Plant pots were placed in containers with freshwater after the three month treatment. Recovery of the plants was assessed by measuring the height of marked stems and the percentage dead material.

Results

Both salinity ($F = 8.10$, $P < 0.005$, $df = 4$) and inundation ($F = 6.99$, $P < 0.005$, $df = 2$) had a significant effect on stem elongation (Fig. 23A). However a two-way ANOVA showed that there was no significant interaction between salinity and the inundation treatment ($F = 1.02$, $P > 0.05$, $df = 8$).

Stem elongation was significantly reduced for the dry treatment (I1) at all salinity levels compared to the tidal (I2) and submerged treatments (I3). Plants grew equally well whether submitted to tidal or completely submerged conditions. This was over three months. Stem elongation was greatest for the 0 ppt submerged treatment. Completely submerged plants grew faster at 0 and 75 ppt compared to the dry and tidal treatments. For all inundation treatments stem elongation was reduced at 55 and 75 ppt. After three weeks there were signs of salinity stress, i.e., leaves were rolled and necrotic.

The number of stems produced per week remained fairly constant for the dry and tidal treatment between 0 and 35 ppt (Fig. 23B). Stem production was reduced at 55 and 75 ppt. Stem production for the submerged (I3) treatment, was significantly lower than for the dry and tidal treatments ($F = 8.99$, $P < 0.001$, $df = 2$).

Leaf elongation (Fig. 24A) was significantly faster for the dry treatment compared to the tidal and submerged treatment ($F = 7.67$, $P < 0.01$, $df = 2$). Leaf elongation decreased as the treatment salinity increased for the tidal and submerged treatment, but for the dry treatment was greatest at 15 and 35 ppt.

Sediment salinity increased with increasing salinity treatment (Fig. 24B). At 0, 15 and 35 ppt, sediment salinity for the dry treatment was higher than for the tidal and submerged treatments. There was no significant difference between the sediment salinity values for the tidal and submerged treatments ($F = 0.22$, $P > 0.05$, $df = 1$).

Dry 15 and 35 ppt treatments were the only plants that showed any signs of recovery after six weeks in freshwater. The percentage above-ground material increased for these plants as new green leaves were produced. The 55 and 75 ppt plants showed no signs of recovery. Approximately 50 % of the above-ground material was necrotic and dead at the end of the three-month saline treatment.

Discussion

Stem elongation, leaf elongation and stem production responded differently to the three inundation treatments. Stem elongation was reduced in the dry treatment, stem production was reduced in the submerged treatment and leaf elongation was greatest in the dry treatment. All three growth parameters responded similarly to salinity, i.e., *Spartina maritima* grew equally well at a salinity level between 0 and 35 ppt but growth was reduced at 55 and 75 ppt. (Plate 6). Plants showed typical signs of stress after three weeks, i.e., leaves were tightly rolled and twisted, with the outer leaves showing signs of chlorosis (Nestler 1977). Woodhouse *et al.* (1974, in Linthurst and Seneca 1981) found that salinity concentrations greater than 45 ppt. caused die-back of *S. alterniflora*. The optimum growth salinity of *S. alterniflora* was estimated between 0 - 20 ppt (Haines and Dunn 1976, Linthurst and Seneca 1980). This study showed that *S. maritima* grew equally well at a salinity of between 0 - 35 ppt over the three-month study period.

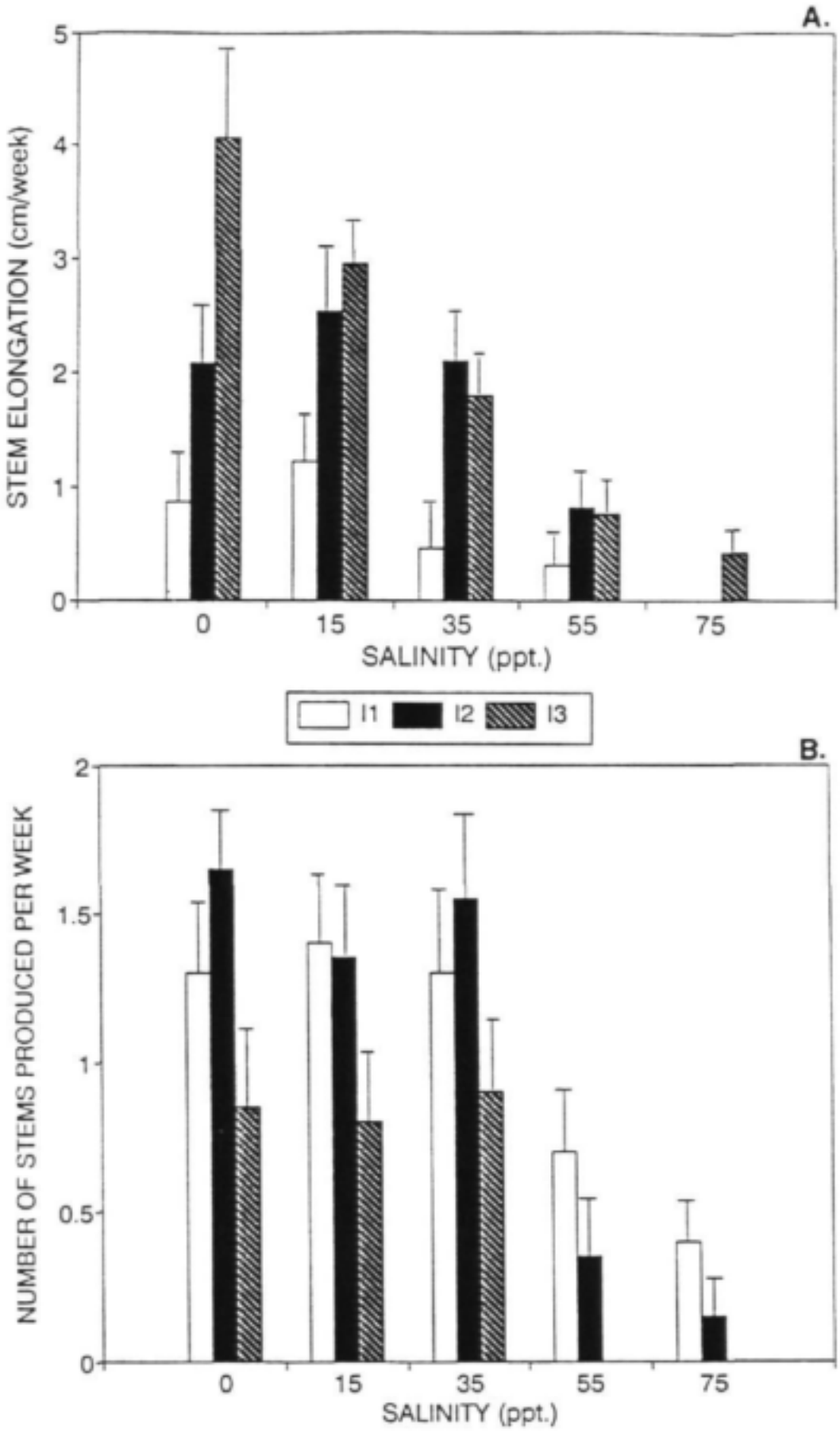


Figure 23 The effect of salinity and inundation on the rate of *Spartina maritima* stem elongation (A) and number of stems produced per week (B) over a three month period.
(Bars = SE, n = 15).

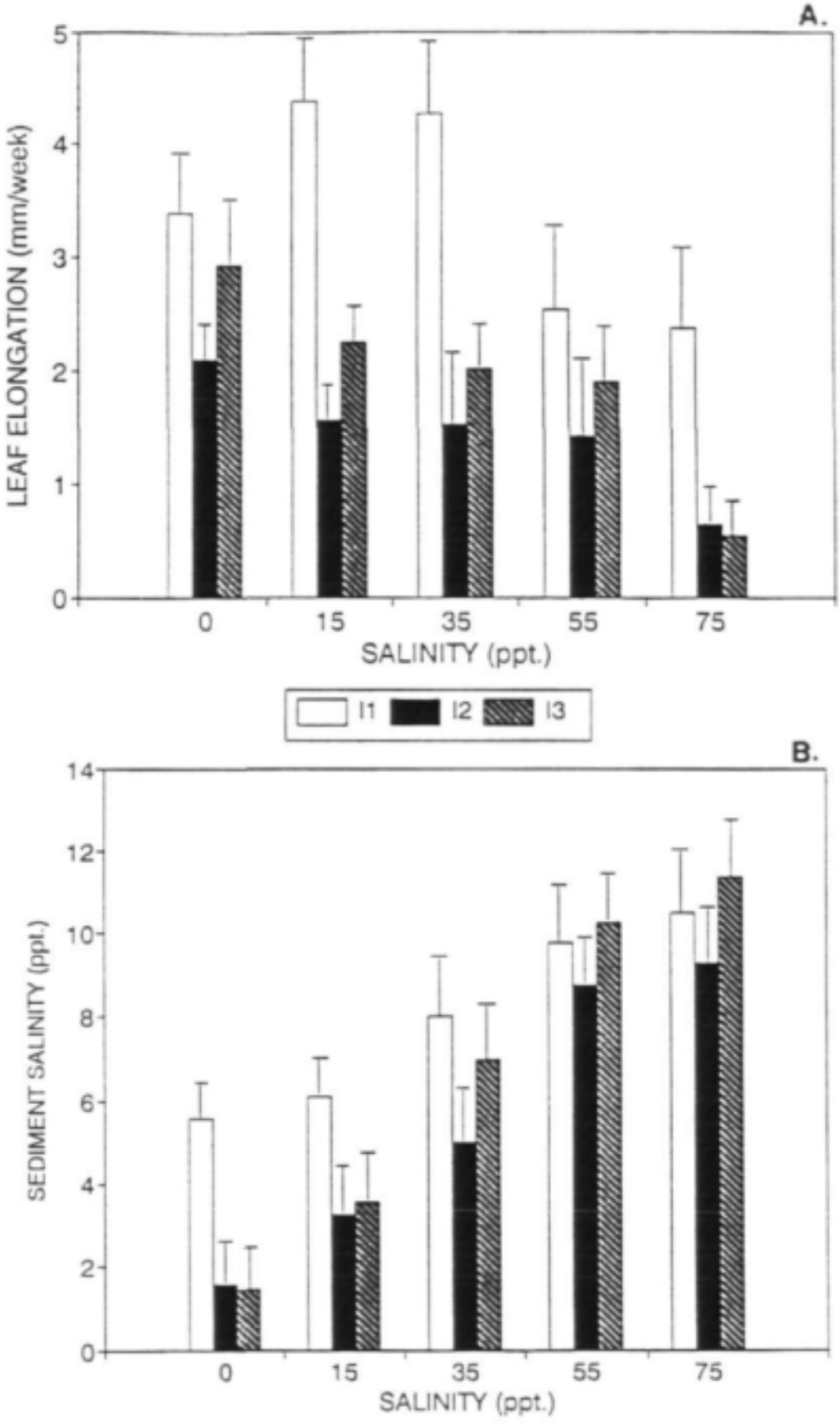


Figure 24 The effect of salinity and inundation on *Spartina maritima* rate of leaf elongation (A) and sediment salinity of *S. maritima* pots (A) treated with 5 salinity levels and 3 inundation treatments over a three month period.

Many studies (Haines and Dunn 1976; Zedler *et al*. 1980, Linthurst and Seneca 1981 in Dame and Kenny 1986) on *S. alterniflora* have shown that growth rates were reduced at higher salinity. At high salinity it is thought that more energy is expended by the plants on metabolic regulation and internal ion balance than on growth process (Haines and Dunn 1976, Dame and Kenny 1986). *Spartina* spp. possess salt glands but salt regulation under conditions of high salinity may be more dependent on salt exclusion by the roots than on secretion by shoots. This may be due to an inability to take up water under these low osmotic potentials or to an impairment of the secretion mechanism itself (Smart and Barko 1980).

The rate of stem elongation was greatest for the completely submerged plants treated at 0 ppt. As mentioned earlier, some species are stimulated to elongate their stems under submerged conditions (Jackson and Drew 1984). This 'depth accommodation' permits wetland plants to emerge quickly so that leaves can function in gas exchange (Kirkman and Sharitz 1993). *Spartina maritima* showed rapid stem elongation, but only under freshwater submerged conditions.

The rate of leaf and stem elongation for *S. maritima* did not differ significantly for tidal or completely submerged plants. *Spartina maritima* can survive periodic tidal submergence for longer periods than other plants and it therefore occupies the lower elevation of salt marshes. This ability to withstand tidal inundation for longer periods than other halophytes enables it to colonize barren intertidal areas (Redfield 1972 in Gammill and Hosier 1992). According to Pearcy and Ustin (1984) *Spartina foliosa* occurred in the low marsh as *Salicornia* was excluded from this area by adverse tidal flooding effects on seedling survival.

The dry treatment inhibited growth of *S. maritima*, suggesting the requirement by the plant for waterlogged conditions. *Spartina alterniflora* thrives in anoxic low marsh habitats due to its ability to oxygenate its roots and rhizosphere (Bertness 1991), the same may apply for *S. maritima*. The plants possess extensive aerenchyma, which are internal gas spaces that extend from the leaves to the root tips (Teal and Kanwisher 1966 in Bertness 1991). Oxygen diffuses to the root zone from the aerial portions of the plant. In South African estuaries *Spartina* is not found in periodically closed estuaries as in these systems the intertidal zone is not well defined. Other plants adapted to drier conditions possibly outcompete *Spartina*. Bertness (1991) used transplant experiments and found that *S. alterniflora* was competitively excluded from the high marsh habitat.

The production of new stems was significantly reduced for the completely submerged treatments compared to the tidal and dry treatments. Anoxic sediment conditions may inhibit stem production or more energy may be allocated into stem elongation under completely submerged conditions. The large allocation to stem elongation is probably necessary to keep the leaves above the water level (Pearcy and Ustin 1984).

Mendelssohn and Seneca (1980) found that under stagnant, standing water conditions growth of *S. alterniflora* was inhibited. Their experiment monitored growth over five months. The combined effects of sea-level rise and subsidence tend to increase plant submergence that often leads to plant death and open waterbodies (Mendelssohn and McKee 1988). In this study, three months of submergence did not affect *S. maritima* stem elongation. Plants were covered with 60 cm of water and approximately 25 % of the aerial parts remained above water (Plate 7). This closely simulated *in situ* high tide conditions. The diffusion of atmospheric oxygen to the roots via the stem was, therefore, not completely blocked (Mendelssohn and Seneca 1980).

Leaves grew faster for the dry treatment compared to the tidal and submerged treatments. Leaf growth is extremely sensitive to flooding and possible root anoxia (Trought and Drew 1980a in Jackson and Drew 1984). Leaf expansion can slow within 20-40 min. of submergence (Wenkert *et al*. 1981 in Jackson and Drew 1984).

The dominant factors that control salinity of salt marsh soils are evaporation, duration of tidal flooding, frequency and amount of rainfall and the salinity of tidal waters (Mahall and Park 1976). Rainfall and freshwater run-off is important as it reduces sediment salinity. Studies have shown that halophytes have increased productivity at lower salinity. In southern California salt marshes, heavy rainfall caused flooding of the marshes and a 40 % increase in the biomass of *Spartina foliosa* was recorded (Zedler 1983). Freshwater input into estuaries is important as it reduces sediment salinity and increases marsh productivity. Daily tidal flushing is important as this study has shown that *S. maritima* stem elongation was reduced when plants were exposed to dry conditions. Reduced freshwater input can cause marine sediments to accumulate in the estuary mouth, shrinking the mouth dimensions and reducing tidal flushing of the marshes. If the mouth of an estuary closes due to reduced freshwater input there is the possibility that *S. maritima* communities may disappear. *Spartina maritima* is absent from periodically closed South African estuaries and this may be attributed to a requirement for tidal flooding and saturated substrates.

Conclusion

Spartina maritima appears to be adapted to its low marsh habitat as it can survive submergence for longer periods than other marsh plants. The rate of stem and leaf elongation did not differ significantly for completely submerged or tidally inundated plants. Growth was reduced for the dry treatments and at salinity levels greater than 35 ppt. *Spartina maritima* is not found in periodically closed South African estuaries. This may be attributed to the plant's requirement for tidal flooding and saturated substrates.

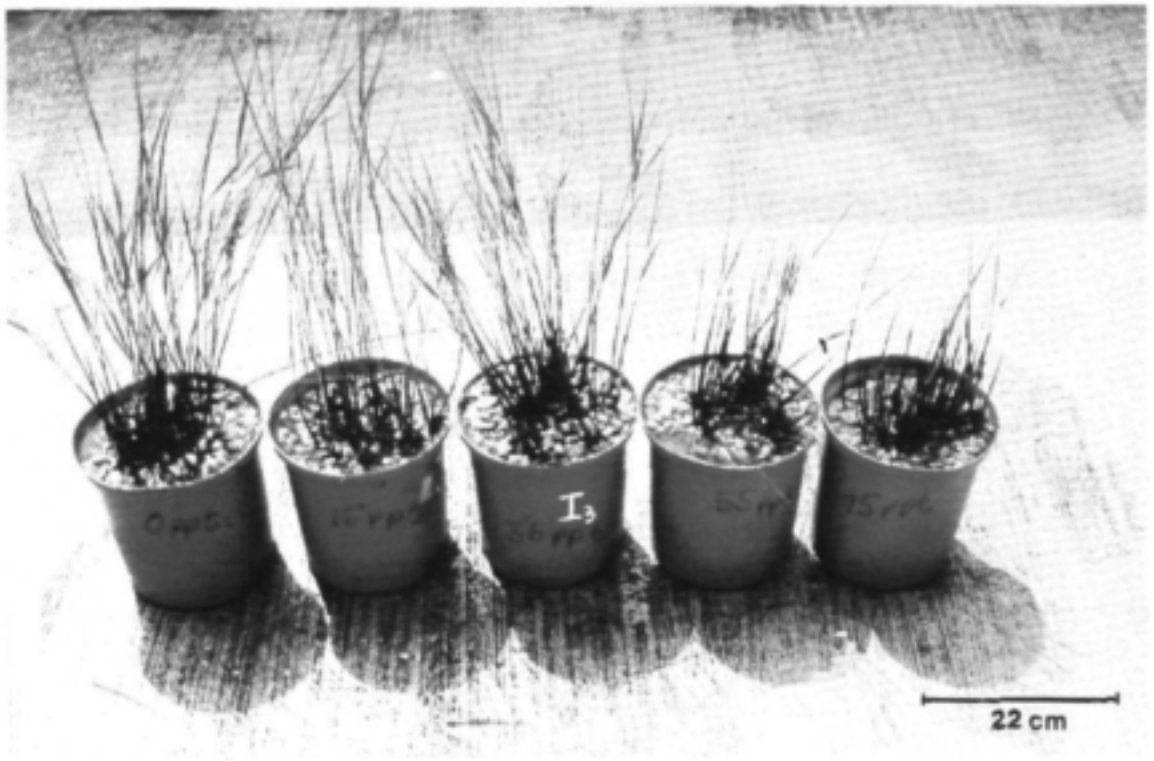


Plate 6 *Spartina maritima* grew equally well at salinity between 0 - 35 ppt. but growth was reduced at 55 and 75 ppt.

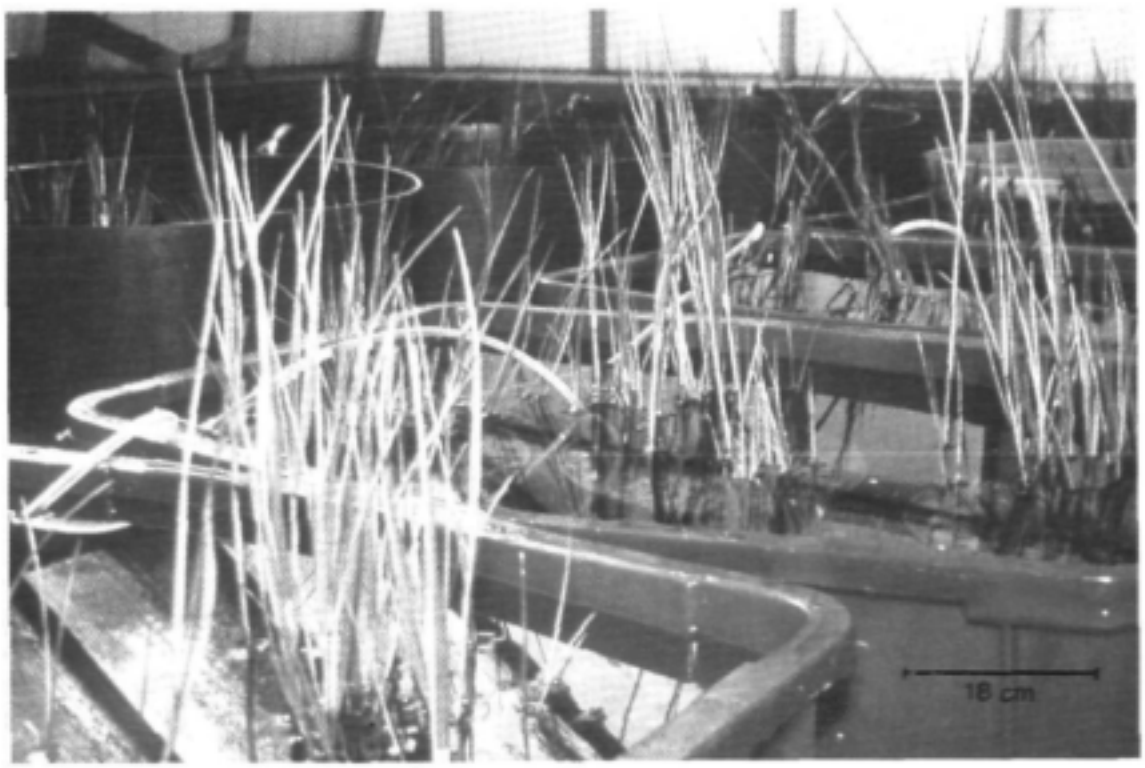


Plate 7 Approximately 25 % of the aerial tissue remained above water for the submerged *Spartina maritima* treatments.

3.3 EMERGENT MACROPHYTES

THE ECOLOGICAL IMPLICATIONS OF TOLERANCE TO SALINITY OF *PHRAGMITES AUSTRALIS* (CAV.) TRIN.

Introduction

The common reed *Phragmites australis* (Cav.) Trin ex Steudel (= *Phragmites communis* Trin.) is a rhizomatous grass species that forms monospecific stands in wet habitats (Hara *et al.* 1993). *Phragmites australis* reeds dominate freshwater wetlands in temperate regions throughout the world (Simpson *et al.* 1983, Hocking 1989), including South Africa (Gordon-Gray and Ward 1971, Day 1981b). They also fringe natural or man-made water bodies (Howard-Williams 1980, Hocking 1989). *Phragmites* forms dense beds in the brackish upper reaches of South African estuaries that have a gradient of decreasing salinity up the length of the estuary (Adams *et al.* 1992). In marine dominated estuaries, salinity is uniform (35 ppt) from the mouth to the head of the estuary. In these systems *Phragmites* beds are found at the confluence of small freshwater streams and seeps flowing into the estuary.

Studies on the biology of *P. australis* have indicated that there is a wide range of variation within the species, depending on both genetic differentiation and phenotypic plasticity (Daniels 1991). *Phragmites* has a wide range of ecotypes, some of which are salt tolerant (Björk 1967, Waisel 1972). According to Nikolaevskii and Smirnova (1968 in Benfield 1984) *P. australis* is a pseudo-halophyte, i.e. a plant that cannot tolerate strong salinization and escapes from it by locating the active part of the root system in deeper and less saline soil levels.

The objective of this study was to determine whether *P. australis* survives in saline estuaries because a) it can tolerate high salinity or b) because its root and rhizome system is located in fresh or only brackish water. Detailed experiments on salinity and waterlogging tolerance as undertaken for *Spartina maritima* and *Sarcocornia perennis* were not conducted for *P. australis* as adequate overseas and local literature was available on the plant's tolerances. Table 5 shows the reported salinity tolerances of the plant. From these data it is apparent that *P. australis* plants include both halophytic and glycophytic ecotypes.

Table 5 Reported salinity ranges for *Phragmites australis*.

REFERENCE:	Gallagher *et al.* 1987 in Hellings and Gallagher 1992
SALINITY:	0 - 22 g l^{-1}
COMMENTS:	intertidal populations, Delaware estuary, Atlantic ocean
REFERENCE:	Matoh *et al.* 1988
SALINITY:	45 g l^{-1}, Na^+ pore water
REFERENCE:	Mills and Gallagher unpublished in Hellings and Gallagher 1992
SALINITY:	50 g l^{-1}, interstitial pore water
	65 g l^{-1}, grown hydroponically in the glasshouse
REFERENCE:	Hellings and Gallagher 1992
SALINITY:	30 g l^{-1}, production 1/5 of freshwater plants
COMMENTS:	glasshouse study over 1 growing season
REFERENCE:	Matoh *et al.* 1988
SALINITY:	20 - 34 g l^{-1}
COMMENTS:	greenhouse 6 weeks, 20 g l^{-1} growth unaffected, growth reduced 34 g l^{-1}
REFERENCE:	Benfield 1984
SALINITY:	30 ppt
COMMENTS:	100 % mortality after 94 days
REFERENCE:	Starfield *et al.* 1989
SALINITY:	growth optimal < 25 ppt, > 46 ppt die back
COMMENTS:	observations St. Lucia estuary.

Phragmites australis has well developed mechanisms of flood tolerance. Armstrong and Armstrong (1990, 1991) have reported evidence of a pressurized convective throughflow of gases which increases oxygenation in the plant rhizomes and surrounding rhizosphere. *Phragmites* has been reported to grow in water up to 4 m deep in Uganda (Haslam 1971). Under these conditions oxygen transport to the below-ground parts becomes restricted resulting in metabolic losses and rhizome mortality. Also more energy would be needed to support the extension of spring shoots to the water surface (Graneli *et al.* 1992). Squires and van der Valk (1992) found that *P. australis* survived for one to two years in water too deep for long-term persistence.

Phragmites australis seeds and buds are sensitive to continual flooded conditions. Buds with attached rhizomes did not emerge from cores flooded to the soil surface (Hellings and Gallagher 1992). Braendle and Crawford (1987) stated that rhizomes could survive anoxic conditions for 28 days, although the buds did not elongate during this time. *Phragmites australis* seedlings (shoot length 3-5 cm) did not show any significant growth when submerged, whereas *Scirpus lacustris* seedlings established and developed underwater (Weisner *et al.* 1993). Seedlings may do poorly when submerged since they do not have sufficient aerenchyma to oxygenate the substrate (Bertness 1991). Weisner *et al.* (1993) found that germination of *P. australis* seeds were inhibited by submergence.

A primary effect of flooding is reduced photosynthesis because terrestrial plants can only use CO_2 as a carbon source, whereas submerged macrophytes can have a high bicarbonate utilization (Schat 1984; Lucas and Berry 1985 in Ernst 1990). According to Sand-Jensen *et al.* (1992) submerged leaves of *Phragmites* can photosynthesize when submerged, although at a very low rate. This is in contrast to Hurliman (1951 in Walker and Waygood 1968) who pointed out that the leaves of *Phragmites* cannot withstand submergence.

Methods

Field studies

The salinity range in which *P. australis* naturally occurs was assessed from an initial survey of Cape Province estuaries.

The relationship between *Phragmites* height and *in situ* salinity was studied in the Kafferkuils and Keurbooms estuaries (Fig. 11). Plates 8 and 9 show the *Phragmites* stands sampled in the two estuaries. At each stand plant height was recorded at a landward, middle and intertidal (seaward) site. Sediment salinity, interstitial and surface water salinity was measured at each site. Surface and bottom (15 cm) sediment samples were collected for salinity measurements. Salinity was measured by adding 25 ml distilled water to 5 g air dried sediment. Samples were mechanically shaken for 30 minutes. The suspension was filtered through Whatman No. 1 paper into a 25 ml volumetric flask. The supernatant was then made up to 25 ml (Smith and Atkinson 1974) and salinity measured using a hand-held Atago refractometer (Labotec). An interstitial water sample was obtained by inserting a syringe into the substrate. The sample was filtered through Whatman No. 1 filter paper and the salinity was measured.

In the Keurbooms and Kafferkuils estuaries leaf fluorescence was measured for seven plants at the landward, middle and seaward sites. The objective was to establish whether differences in leaf fluorescence could be attributed to differences in *in situ* salinity.

Plate 8 Tidal inundation of *Phragmites australis* in the Kafferkuils estuary.

Plate 9 The *Phragmites australis* stand in the Keurbooms estuary.

Laboratory studies

Laboratory studies investigated the effect of increased salinity on the plant root and rhizome system. Spring buds of *P. australis* with attached rhizomes were collected from the Seekoei estuary and placed in 30 pots with sediment obtained from the collection site. Substrate and interstitial salinity levels were manipulated by attaching a perforated plastic pipe to the bottom of the plant pot. Plants were repotted into these pots after one month growth in the glasshouse. The perforated pipe was attached to a water holding tank that supplied either freshwater or 20 ppt to the root and rhizome system (Fig. 25). These pots were placed in larger containers that were filled with saline (35 ppt) water for 6h a day. This simulated the possible field conditions where the plant root and rhizome system is located in freshwater while the above ground plant parts are exposed to saline tidal water.

Weekly measurements were made of plant height and the number of dead and alive leaves. This was recorded for 15 short (height: approximately 30 cm) and 15 tall plants (height: approximately 60 cm) for both the saline and freshwater treatment. Plant height was measured from the sediment surface to the base of the uppermost leaf. Daily measurements were made of leaf fluorescence (Fv/Fm ratio).

Fluorescence was measured for leaves of different insertion number to determine whether leaf age significantly affected leaf fluorescence. Six replicate plants were measured and the average number of leaves per shoot was eight.

Results

Field studies

In the Kafferkuils estuary surface, interstitial and sediment salinity decreased from the seaward to the landward site. Except for the middle site the interstitial salinity was lower than the surface salinity (Fig. 26). In the Keurbooms estuary surface salinity was the same (34 ppt) at all three sites (Fig. 26). The area where *Phragmites* occurred was not as steep as in the Kafferkuils estuary. At high tide the plants at the landward site were inundated with tidal water. In all cases interstitial water salinity was lower than surface water, indicating that *Phragmites* did occur at a site of freshwater seepage.

Figure 27 shows that the height of *P. australis* decreased significantly from the landward to the seaward site in the Kafferkuils ($F = 38.9$, $P < 0.0001$, $df = 2$) and Keurbooms estuaries ($F = 106.5$, $P < 0.0001$, $df = 2$). Leaf Fv/Fm values at the different sites did not differ significantly for plants in both the Kafferkuils ($F = 0.34$, $P > 0.05$, $df = 2$) and Keurbooms estuaries ($F = 0.66$, $P > 0.05$, $df = 2$).

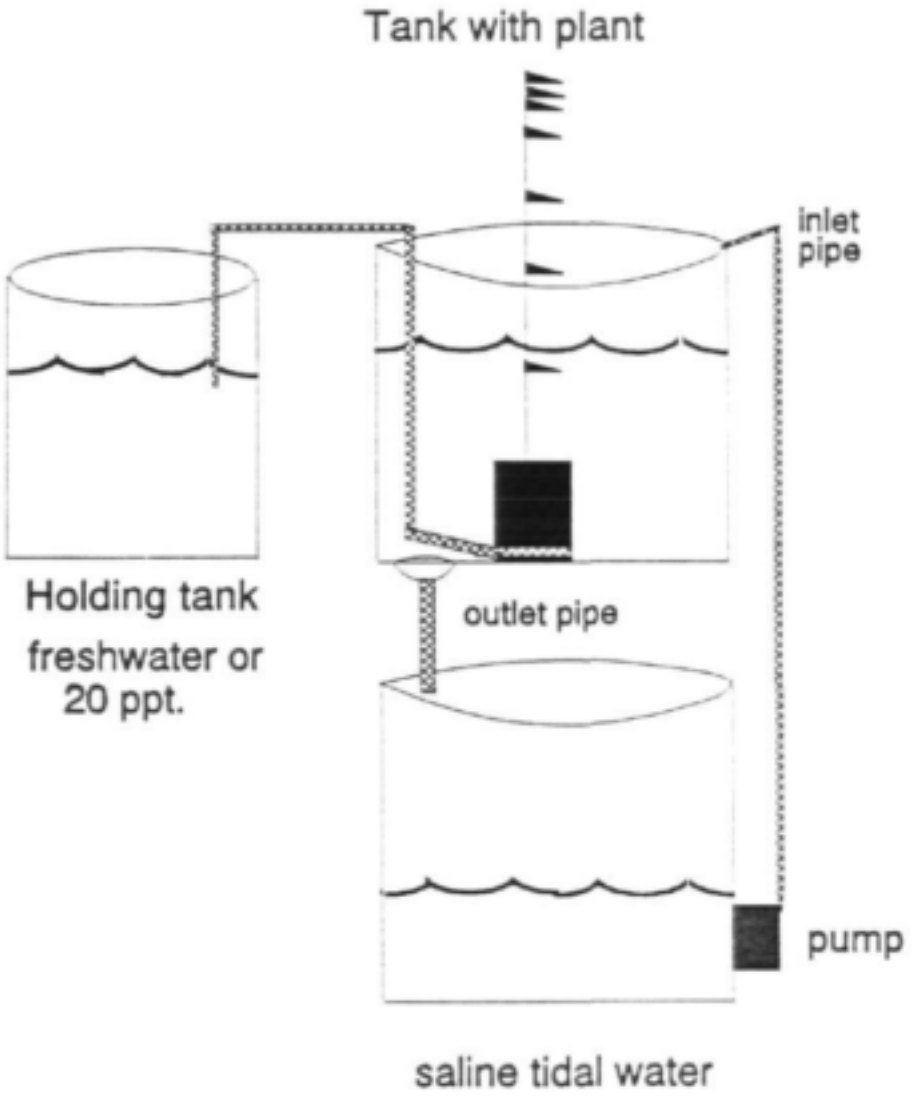


Figure 25 The diagram shows the 3-tank system used to simulate *in situ* conditions for *Phragmites australis*. The roots and rhizomes were bathed with either 20 ppt or freshwater. The plants were inundated for 6h daily with tidal saline (35 ppt) water.

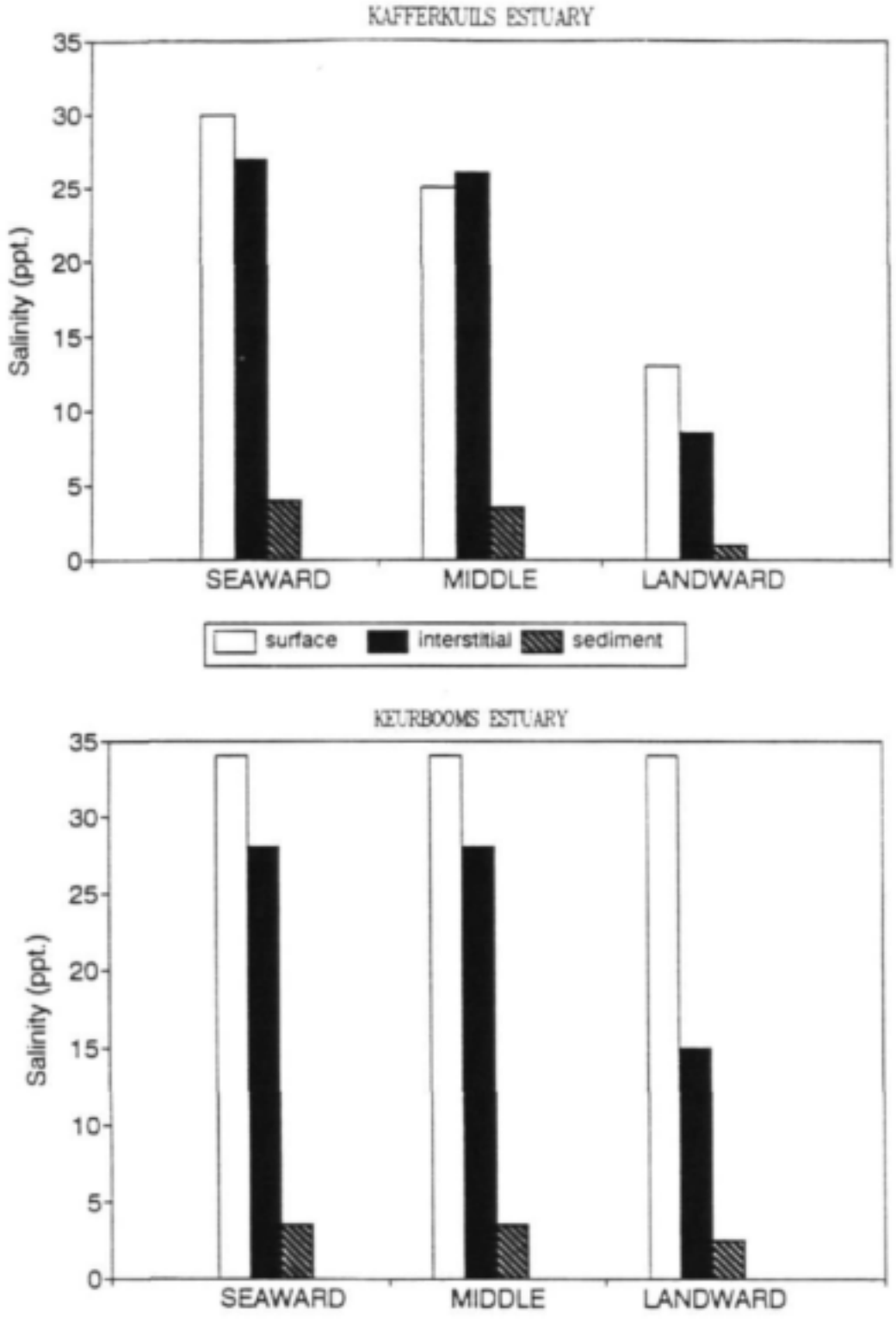


Figure 26 Surface, interstitial and sediment salinity at three sites in the *Phragmites* stand in the Kafferkuils and Keurbooms estuaries.

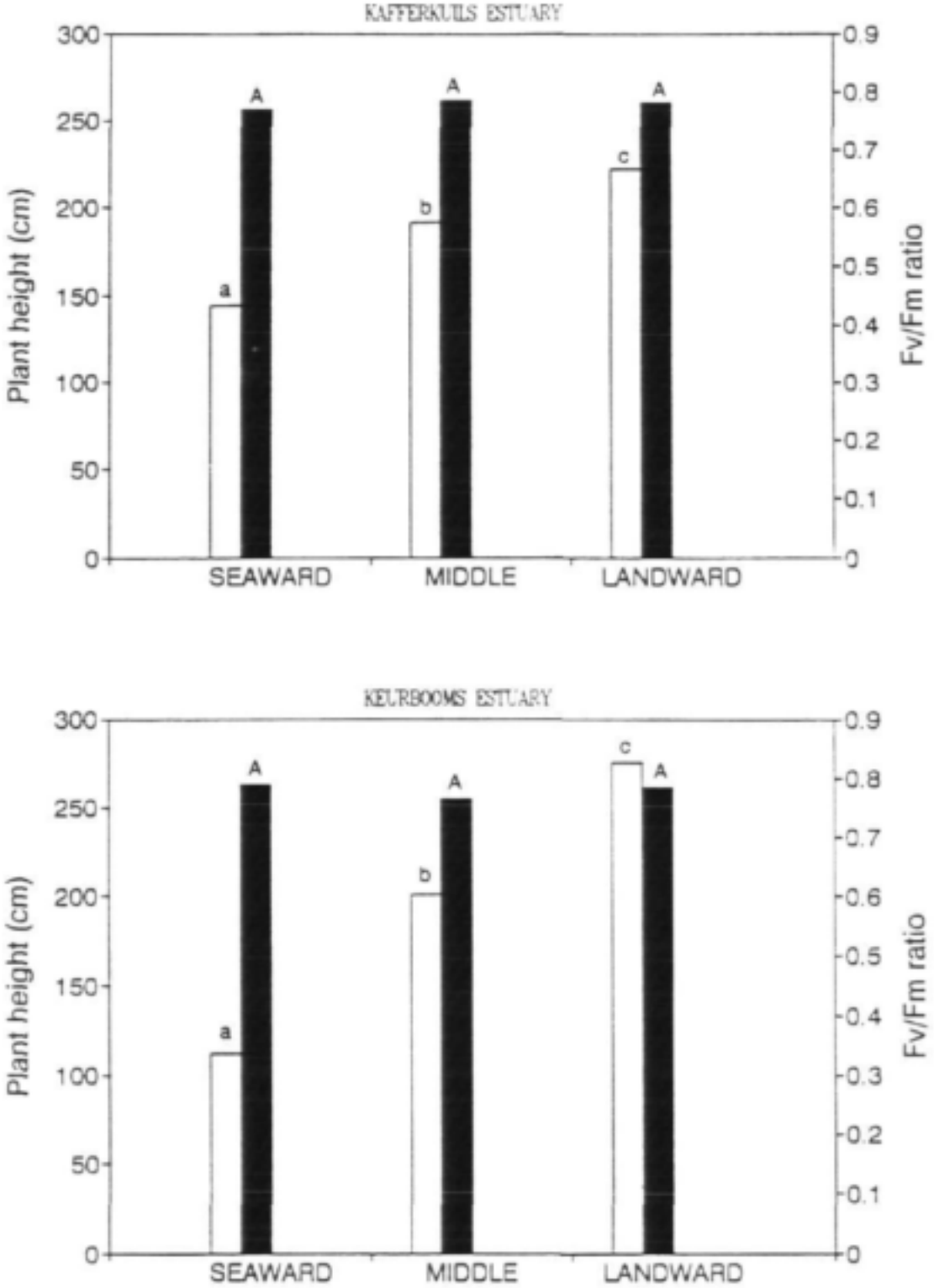


Figure 27 *Phragmites australis* height and leaf Fv/Fm values for three sites in the Kafferkuils and Keurbooms estuaries. Means with different letters (a-c) were significantly different at $P \leq 0.05$ using the Student Newman-Keuls multiple range test.

Laboratory studies

The difference in Fv/Fm readings between individual leaves of the insertion numbers 1 to 8 were small (Fig. 28). Fv/Fm values ranged between 0.816 and 0.838. The 2nd or 3rd leaf (insertion no. 6/7) from the shoot apex was used for subsequent fluorescence measurements.

Figure 29 shows that after the 2nd day of treatment Fv/Fm values for the plants treated with saline water were lower than the freshwater plants. Fv/Fm ratios for the plants treated with 20 ppt decreased steadily over time. By the second week the Fv/Fm ratio had decreased to 0.7 showing that the plants were stressed.

After the second week of treatment stem elongation was significantly reduced for both the short plants ($F = 4.54$, $P < 0.05$) and tall plants ($F = 5.04$, $P < 0.05$) treated with 20 ppt (Fig. 30A). Stem elongation of the tall plants was reduced from 4 cm $week^{-1}$ to less than 1 cm $week^{-1}$. There was no reduction in stem elongation for the tall and short plants treated with freshwater after the second week. Over the four week period these plants grew at approximately the same rate, although growth of the short plants was slower (Fig. 30A).

The percentage dead leaves increased steadily over time for the short and tall plants treated with 20 ppt (Fig. 30B). The short plants were more sensitive to the saline treatment compared to the tall plants. By week four 90 % of the leaves were dead compared to 73 % for the tall plants.

The percentage dead leaves of the tall plants treated with freshwater increased over the first two weeks but thereafter remained constant. The shorter plants had a higher percentage of dead leaves compared to the tall plants (Fig. 30B). During daily tidal emergence the shorter plants had most of its leaves underwater. The saline (35 ppt) tidal treatment affected the older leaves that became necrotic and died. However new leaves were being produced all the time and therefore the percentage of dead leaves between week two and four remained constant (approximately 40 %).

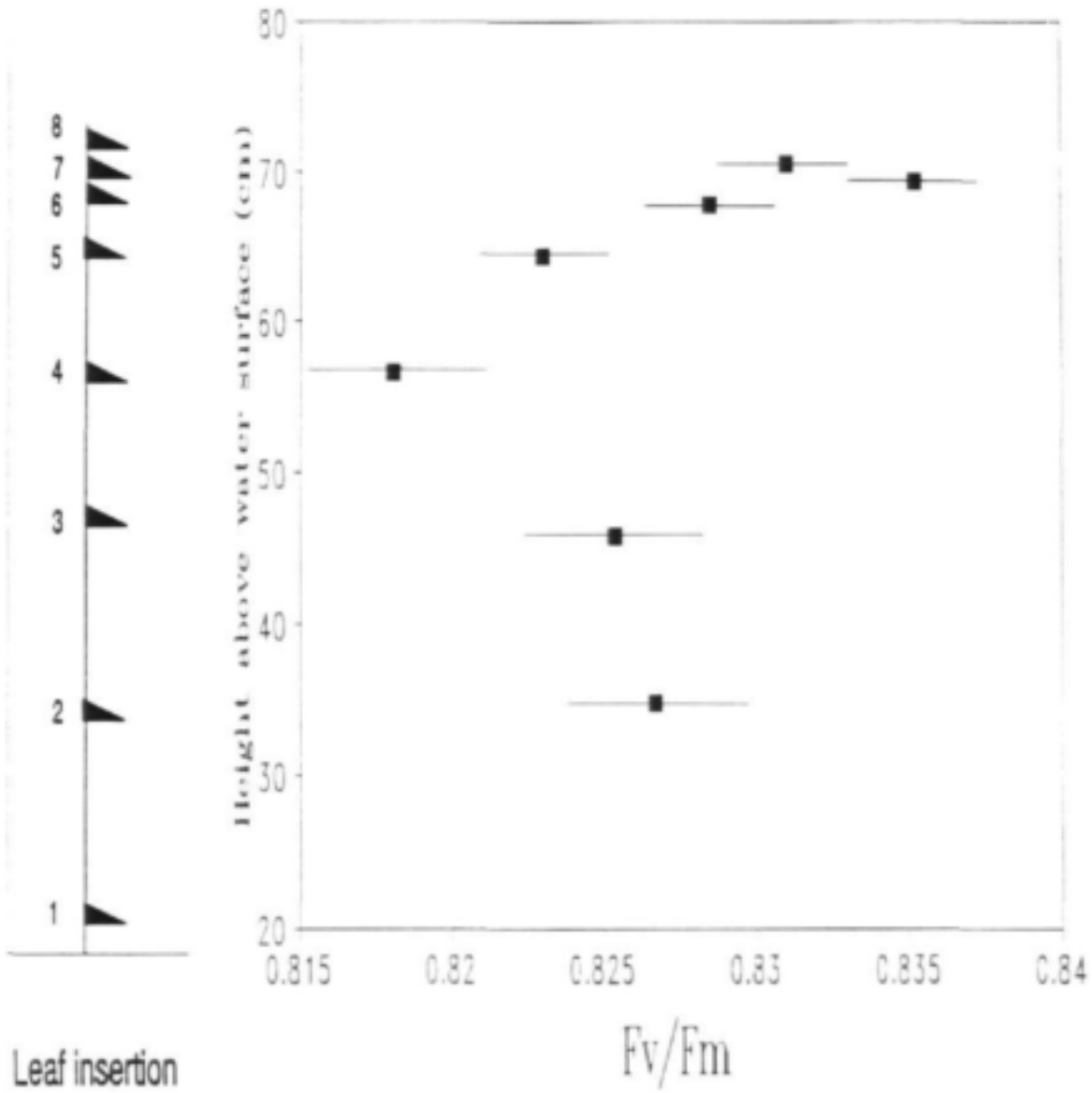


Figure 28 Vertical distribution of *Phragmites australis* leaf insertion (leaves numbered from base) and corresponding Fv/Fm values. Standard errors are depicted as horizontal bars (n = 6).

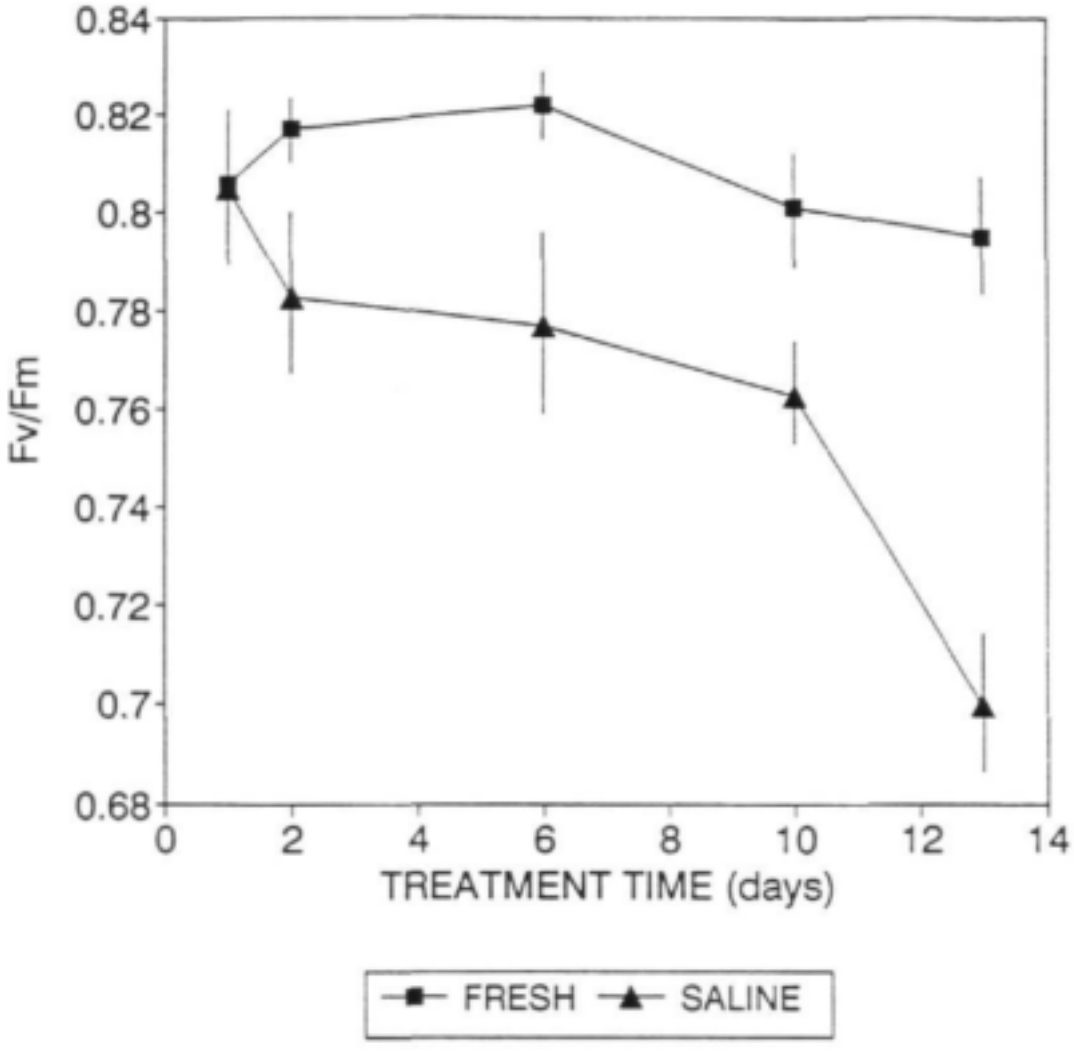


Figure 29 The effect of a fresh and saline (20 ppt) treatment on daily *Phragmites australis* leaf Fv/Fm values.

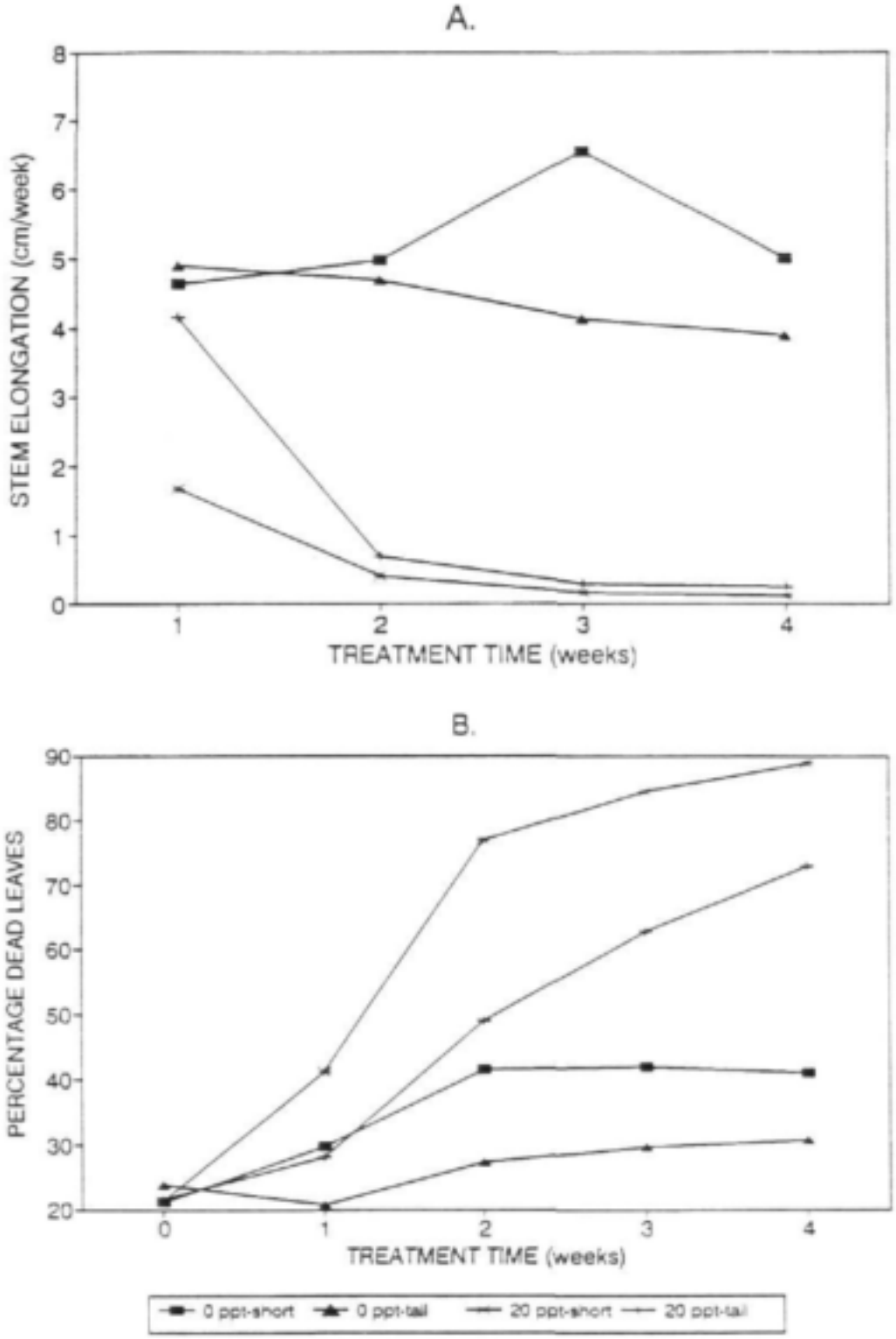


Figure 30 The effect of a fresh and saline (20 ppt) treatment on (A) *Phragmites australis* weekly stem elongation and (B) the percentage of dead leaves compared to the total number of leaves.

Discussion

In the Kafferkuils and Keurbooms estuaries, the height of *Phragmites* decreased from the landward to the intertidal sites. This is likely related to the increase in substrate and interstitial salinity. Hellings and Gallagher (1992) showed that *P. australis* plants grown at 22 ppt were shorter than plants grown in freshwater. Matoh *et al.* (1988) found *Phragmites* plants growing where the soil water was 45 g l^{-1} but the plants were reduced in height (1 m). The reduction in *P. australis* stem elongation and leaf production under saline conditions could be due to the diversion of energy from active meristematic growth to the maintenance of osmotic balance (Hellings and Gallagher 1992).

Plant height decreased away from the water's edge and this was associated with an increase in interstitial and substrate salinity. However, an increase in salinity did not affect leaf fluorescence. The Fv/Fm ratio gives an estimate of photosynthetic capacity, so although growth was reduced the plants still photosynthesized optimally. Pearcy and Ustin (1984) studied the effect of salinity on growth and photosynthesis of three marsh species and found that relative growth rate was more sensitive to salinity than photosynthetic rate. Long term studies found that growth declined more than did photosynthesis. It would appear that salinity affects carbon assimilation per plant via a smaller leaf area rather than a reduced rate of photosynthesis (Munns 1993).

It seems likely that the variability in *Phragmites* height from the landward to seaward sites cannot be accounted for solely by salinity. Sediment type and nutrient availability may also be important. *Phragmites* may favour sites of freshwater seepage as these run-off channels may be rich in nutrients (Lewis, pers. comm.). Soil fertility interacts with salinity to affect apparent increased salinity tolerance. For example the K^+ ion and the Ca^{2+} ion are known to ameliorate salinity stress (Lewis *et al.* 1989, Hawkins 1992).

Although *P. australis* may consist of both halophytic and glycophytic ecotypes, a survey of Cape Province estuaries showed that *Phragmites australis* consistently occurred in estuaries where the salinity was less than 30 ppt. These plants generally form dense communities in the upper low salinity reaches of estuaries. In estuaries where *P. australis* was tidally inundated with seawater (35 ppt) the plants always appeared to occur at sites of freshwater seepage.

In March-April 1991 extended drought conditions had caused high salinity levels in the upper reaches of several east coast estuaries. Salinity was 43 ppt in the upper reaches of the Kariega estuary (Fig. 11) and the above ground portion of the reed *Phragmites australis* was dead. It would appear that the salt tolerant plants *Zostera capensis* and *Spartina maritima* have recently colonized the upper reaches of the estuary. In estuaries with a salinity gradient up the length of

the estuary these plants would be restricted to the lower marine end of the estuary. However, because the saltwater/freshwater interface has moved further upstream, halophyte communities have displaced the brackish communities.

Although above ground parts of *Phragmites* were dead, below ground plant parts (i.e., roots and rhizomes) may be dormant as a freshwater pulse into the estuary could induce new growth. Sometimes salinity levels can be so high and lethal that the root stocks are also killed and then the plants take years to re-establish or never return to the same area. This occurred at False Bay in Lake St Lucia (Ward 1976).

The salinity tolerance of the reed is dependent not only on the level of salinity but also on the duration of the salinity regime (Clucas 1980 in Benfield 1984). Benfield (1984) reported 100 % mortality after a period of 94 days for *P. australis* plants treated with 30 ppt. This study showed that after two weeks the plants subjected to 20 ppt were stressed. Stem elongation and leaf fluorescence (Fv/Fm) were reduced after two weeks. The increase in the percentage of dead leaves showed that younger (shorter) plants were affected more rapidly than older plants.

Conclusion

In many South African estuaries *Phragmites australis* occurs where small tributaries and streams enter the estuary. The plants extend into the intertidal zone where they are tidally inundated with saline water (35 ppt). The laboratory studies showed that *P. australis* can survive tidal inundation with saline water if the roots and rhizomes are located in freshwater. Plants which were supplied with 20 ppt to the roots showed signs of stress after two weeks.

In the Kafferkuils and Keurbooms estuaries plant height decreased away from the water's edge and this was associated with an increase in substrate salinity. An increase in substrate salinity did not affect leaf fluorescence, although growth was reduced the plants still photosynthesized optimally.

4. THE IMPORTANCE OF FRESHWATER FOR ESTUARINE PHYTOPLANKTON

Introduction

Both local (Allanson and Read 1987, Hilmer and Bate 1990, 1991) and overseas studies (Flint 1985, Drinkwater 1986, Cloern 1991b, Paerl *et al.* 1990) have shown that phytoplankton biomass in estuaries is positively correlated with freshwater input. In South Africa, studies on the relationship between freshwater input and phytoplankton response include Allanson and Read's (1987) work in the Kariega, Keiskamma and Great Fish estuaries and Hilmer and Bate's work in the Swartkops and Sundays estuaries (Hilmer 1984, Hilmer 1990, Hilmer and Bate 1990, 1991). The general conclusions from these studies were that in estuaries with large catchments, adequate freshwater inflow supported phytoplankton communities and a pelagic food-chain. Submerged macrophyte communities were dominant in smaller estuaries with reduced freshwater input. Furthermore, flooding usually produced peaks in phytoplankton biomass and production.

Freshwater input supported phytoplankton communities as it brought in nutrients and maintained stable stratified conditions. Strong vertical and horizontal salinity gradients were maintained in open mouth estuaries with adequate tidal exchange. In the Sundays estuary dinoflagellates formed strong blooms during extended neap tides when salinity stratification was most pronounced. The blooms were dispersed on the following spring tide due to vertical mixing (Hilmer and Bate 1991). Previous studies had established the relationship between freshwater input and phytoplankton response for eastern Cape estuaries, therefore the aim of this study was to test these responses in other South African estuaries.

Study area

The estuaries studied included the Berg, Palmiet, Kafferkuils, Gouritz, Groot Brak, Keurbooms, Gamtoos and Sundays (Fig. 31). Physical characteristics of the estuaries sampled are summarized in Table 6. The Berg is a permanently open estuary on the west coast of South Africa. During summer months strong salinity stratification is evident. However during winter the estuary functions as a river mouth because of the high winter rainfall in the catchment. The Palmiet is a small permanently open blackwater estuary (length approximately 1.7 km). The river enters the estuary as a fast flowing mountain stream (Taljaard and Largier 1989). During summer (dry season) the estuary functions as a highly stratified strongly marine system with nutrients being imported from the sea. During winter it is mostly a riverine system with freshwater predominance

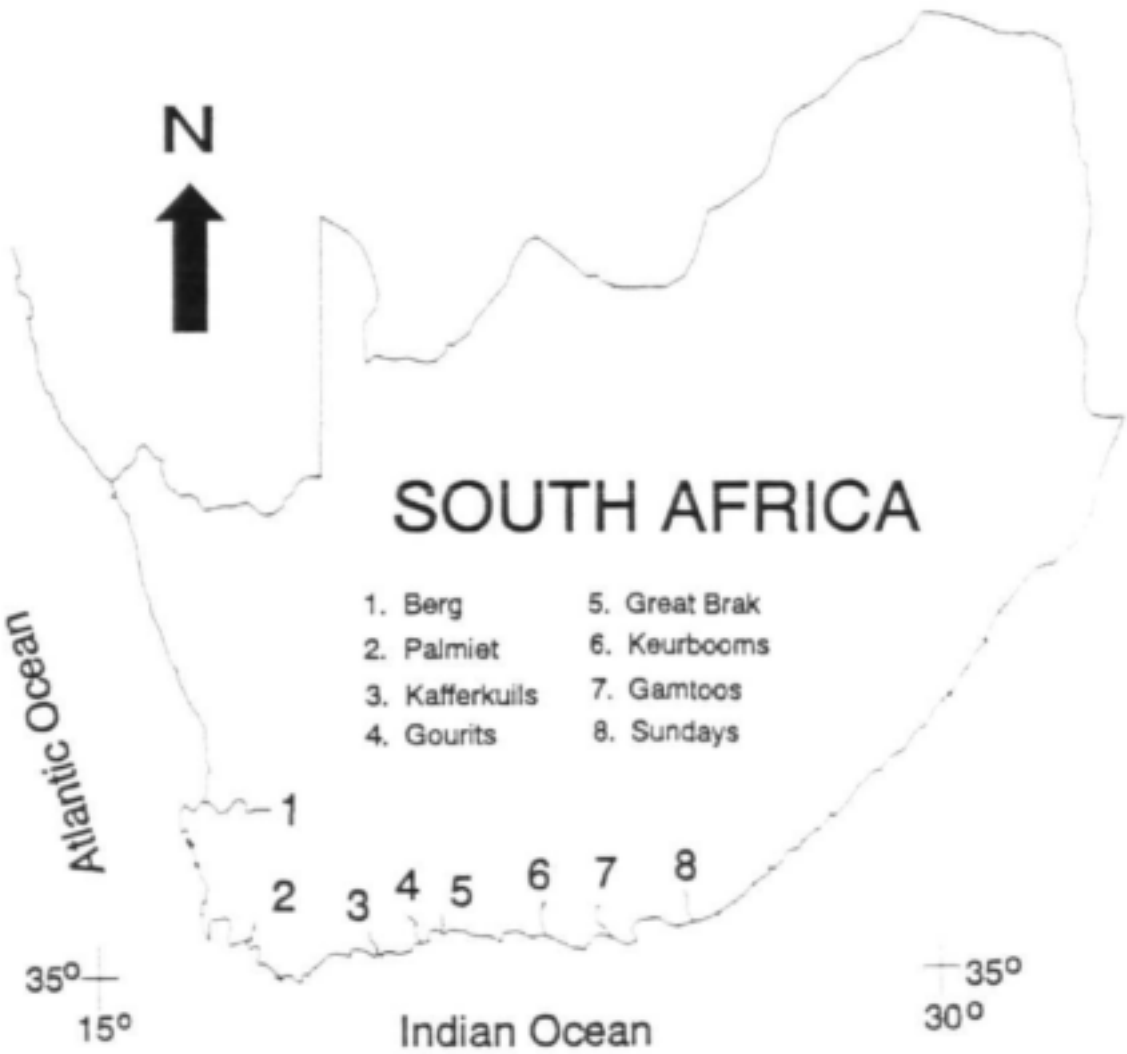


Figure 31 Location of estuaries sampled in August and November 1992 for phytoplankton studies.

Table 6 Physical characteristics of estuaries sampled for phytoplankton study. (Anon. 1987/1988, NRIO data reports D8703, D8705, D8706 and D8802).

Estuary	Catchment area (km^2)	River length (km)	Mean Annual Run-off (m^3x10^6)
BERG	7715(km)	294.3	1035.47
PALMIET	535	72.5	200.92
KAFFERKUILS	1550	67.4	106.42
GOURITZ	45715	416	539.05
GREAT BRAK	190	31.5	38.79
KEURBOOMS	1080	85.3	176.86
GAMTOOS	34635	644.8	502.51
SUNDAYS	20990	481.0	202.26

The Kafferkuils is a permanently open estuary but has a constricted tidal inlet. The major part of the catchment consists of privately owned farms where mixed farming is practised (Carter and Brownlie 1990). Although the Gouritz has a large catchment area, the catchment is situated in an area of low rainfall (Heydorn 1989). The catchment area drains large areas of the Great Karoo and the whole of the Little Karoo, which is an important area for sheep and goat farming. The estuary extends about 8 km upstream of the mouth. During periods of low flow it is a marine dominated system.

The Great Brak is a temporarily open blackwater estuary. This estuary has become the focus of interest because of the recent construction of the Wolwedans Dam. Water release and mouth opening is managed by EMATEK (Division of Earth, Marine and Atmospheric Science and Technology) in association with the Great Brak River Environmental Committee.

The Keurbooms is a permanently open estuary, that at present has no dams in its catchment. The management of the highly dynamic mouth of the Keurbooms estuary is a subject of controversy (Duvenage and Morant 1984). State forest covers part of the catchment. The Gamtoos is a permanently open turbid estuary with a meandering channel deeply eroded into the wide river floodplain. Extensive agriculture takes place in the catchment area (Heinecken 1981). Land use in the permanently open Sundays estuary is solely agricultural, being mostly sheep farming, with some intensive citrus cultivation. The estuary is tidal for about 24 km.

Methods

Sampling was conducted in August and November 1992 in the specified Cape estuaries to establish the relationship between nutrient concentrations and phytoplankton biomass. Low chlorophyll-*a* levels were found in the estuaries during sampling in August. To test whether the high chlorophyll-*a* in east Cape estuaries was related to nutrient input, the nutrient status and phytoplankton levels in two east Cape estuaries i.e. the Sundays and Gamtoos estuaries, were also sampled.

Sampling was carried out at 4 stations in each estuary. At each station water samples were collected using a weighted narrow-necked 1 litre bottle sampler. Water for chlorophyll-*a* and nutrients was collected at 0.5 m intervals from the surface to 1 m depth, and then at 1 m intervals thereafter. All other measurements were made at 0.5 m intervals from the surface to the bottom of the estuary. Salinity and temperature were measured using a WTW model LFG 191 conductivity/temperature meter. Irradiance was measured using a Li-Cor LI 192S submersible quantum sensor and for each depth profile the light attenuation coefficient was calculated.

Water column chlorophyll-*a* was measured by filtering 500 ml of water onto Whatman GF/C filters. The chlorophyll was extracted with 10 ml of 95 % ethanol for at least 2 hours. After extraction, the samples were cleared by filtration. The absorbances were measured at 665 nm before and after acidification with 0.1N HCl in a Milton Roy Spectronic Mini 20. Chlorophyll-*a* concentrations were calculated according to Nusch (1980).

Water samples were collected for phytoplankton identification and cell number counts. The water samples were fixed with buffered formaldehyde, stained with Rose Bengal and settled in settling chambers. Counts were made using a Zeiss inverted microscope and species identified.

Water samples were stored in a cooler box and nitrate-N measured within 24 h using a method adapted from Bate and Heelas (1975).

Results

The horizontal salinity gradients as depicted in Figure 32A (August) and Figure 32B (November) were calculated as the difference between the mean salinity at the mouth and at the head of each estuary. The expert system rule states that if this difference is greater than 10 ppt then a horizontal salinity gradient exists. All estuaries therefore displayed a

horizontal salinity gradient in both August and November. Salinity stratification was calculated as the difference between the mean top (< 0.5 m) and mean bottom (> 0.5 m) salinity. The expert system states that if this difference is greater than 5 ppt then a vertical salinity gradient exists. Vertical salinity gradients were present in all estuaries in August but only in the Gouritz estuary in November.

Although the estuaries sampled had both vertical and horizontal salinity gradients in August, Figure 33A indicates that high chlorophyll-*a* levels (> 20 μg l^{-1}) are better related to water column nitrate concentration than to salinity gradient. A salinity gradient serves as an index of the freshwater input into an estuary, but phytoplankton require high nutrient inputs in order to bloom. Mean chlorophyll-*a* in the Sundays estuary was 29 μg l^{-1} in August 1992. This estuary also had the highest mean nitrate concentration i.e. 106 μg l^{-1}. The difference in nitrate concentration between the head and the mouth was 200 μg l^{-1} indicating the freshwater source of nutrient input. In the Berg estuary the highest chlorophyll-*a* concentrations were also associated with the highest nitrate concentrations (Fig. 33B). However the highest chlorophyll-*a* was found in the mouth of the Berg estuary while the nitrate was associated with freshwater input. The phytoplankton occurring here were probably the result of a flood tide bringing phytoplankton into the estuary from the nutrient rich water of the west coast. We could see that the water at the mouth where the samples were taken was quite distinctive - it was not estuary water.

Although the mean chlorophyll-*a* levels in the Gamtoos estuary in August (Fig. 32A and 33A) were low (~ 5 μg l^{-1}), previous sampling in 1990 established that phytoplankton blooms do occur but mainly in the summer months (Hilmer, pers. comm.). The blooms are located in the stratified water column of the middle reaches of the estuary and can persist for up to one month.

Low chlorophyll-*a* (< 1 μg l^{-1}) was found in the Palmiet and Gouritz estuaries (Fig. 32B). The Palmiet is a well flushed system, water residence time is reduced (Taljaard and Largier 1989) and phytoplankton biomass is therefore low.

Rainfall and freshwater run-off increased between August and November 1992 along the Cape south coast. Comparison between August and November data for the Keurbooms and Great Brak estuaries (Table 7) showed that an increase in freshwater input caused a decrease in mean salinity, an increase in the horizontal salinity gradient, an increase in nitrate concentration and an increase in chlorophyll-*a* concentration. In the Gouritz estuary the mean salinity in November was higher than in August and there was only a slight increase in nitrate concentration from August to November (Table 7). Chlorophyll-*a* concentrations were therefore low (< 1 μg l^{-1}) for both months.

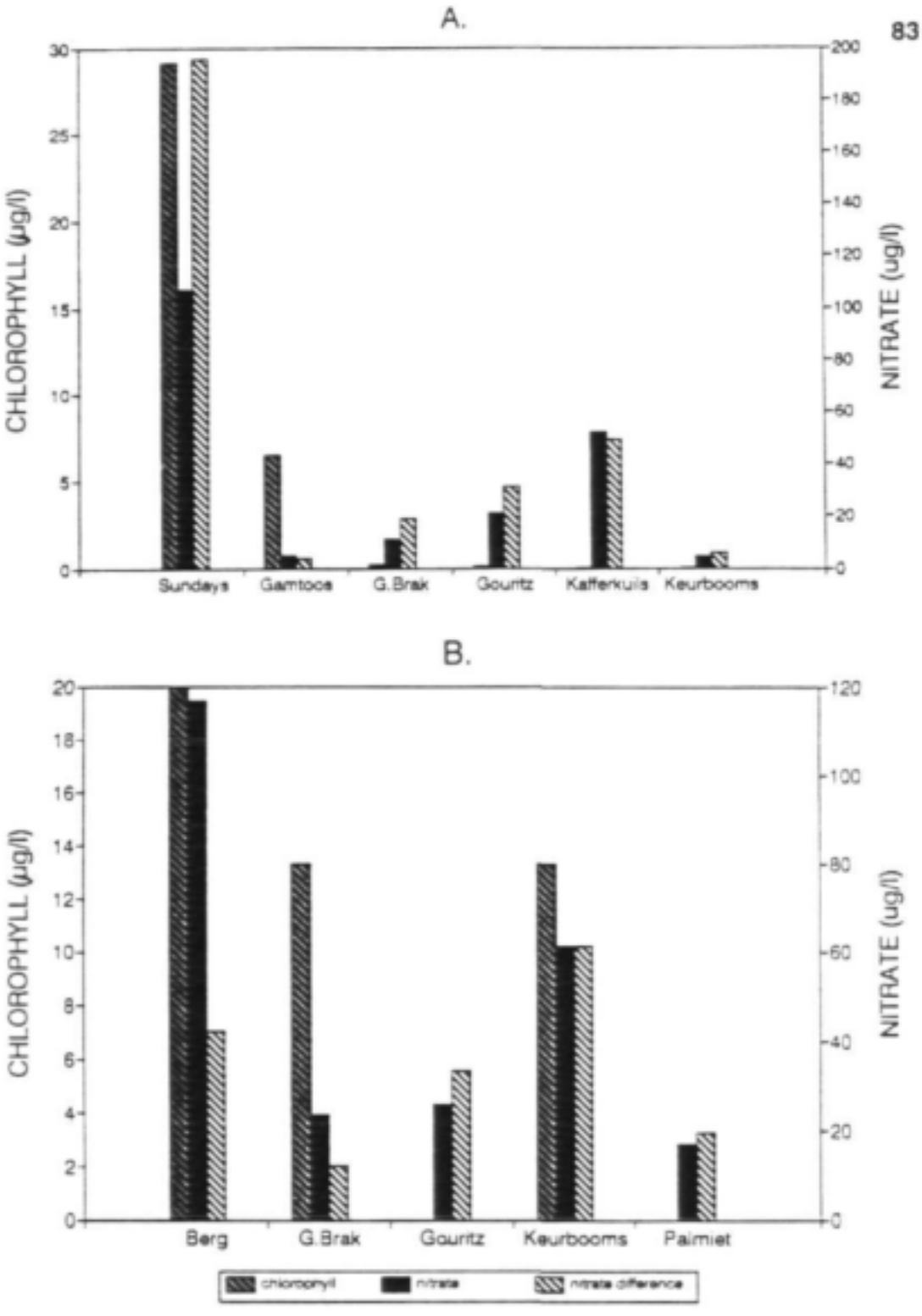


Figure 32 The relationship between chlorophyll-*a*, horizontal salinity gradient and vertical salinity stratification for estuaries sampled in August (A) and November (B).

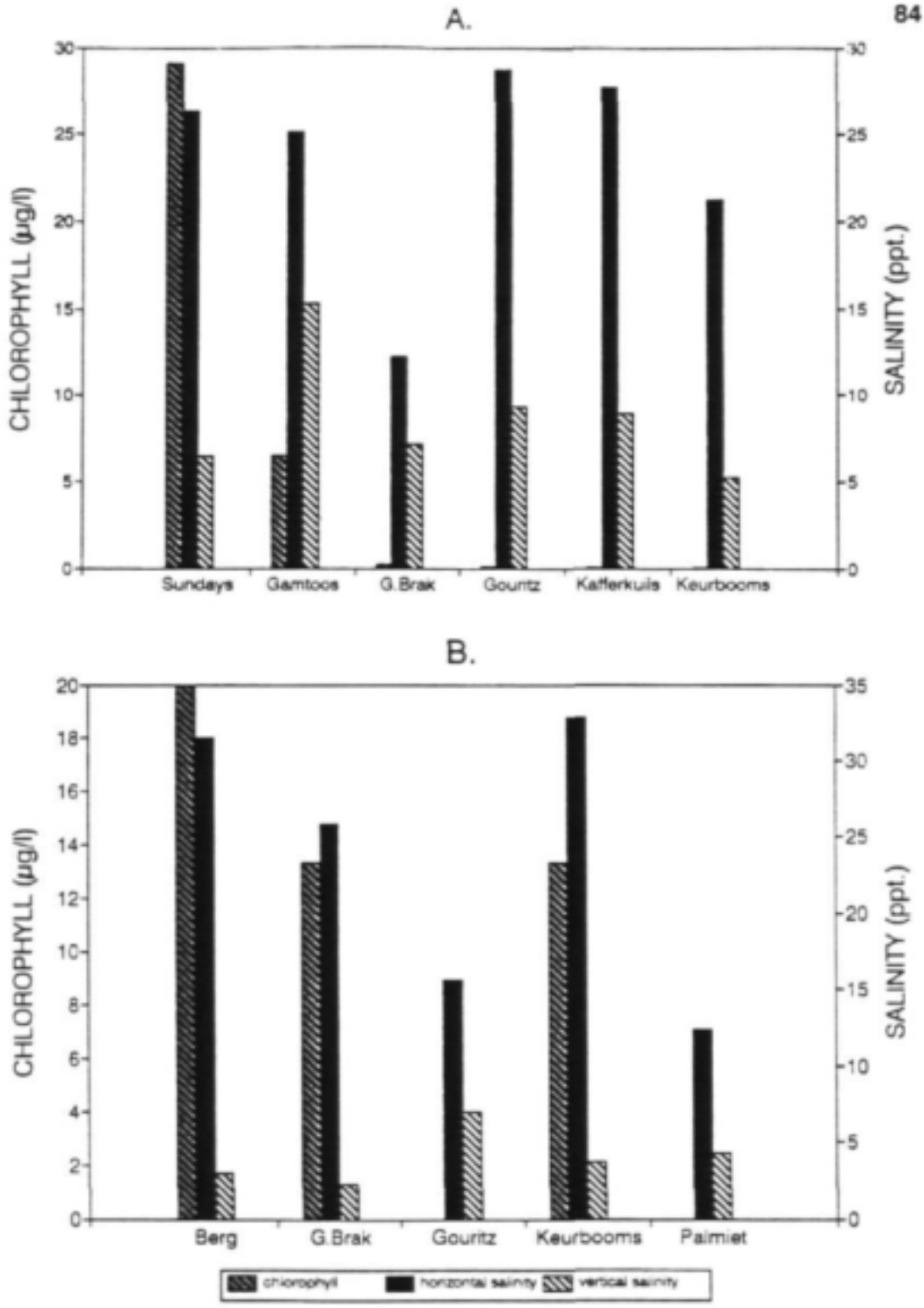


Figure 33 The relationship between chlorophyll-a, nitrate concentration and the difference between head and mouth nitrate concentration for estuaries sampled in August (A) and November (B).

The cells in the water samples taken from the different south coast estuaries were enumerated into groups. The identification of the groups to species level will take some time since Dr G. Boalch (pers. comm.) believes there could be a large number of undescribed species among them. In general, flagellates were the dominant group in all estuaries sampled. The Great Brak estuary also had a number of blue-green algae. Dominant diatom species included *Extubocellulus spinifera* in the Gouritz estuary and *Nitzschia seriata* in the Keurbooms estuary.

Table 7 The difference between August (Aug.) and November (Nov.) chlorophyll, salinity and nitrate concentrations in the Keurbooms, Great Brak and Gouritz estuaries.

	GREAT BRAK		GOURITZ		KEURBOOMS	
	Aug.	Nov.	Aug.	Nov.	Aug.	Nov.
Mean chlorophyll-a ($\mu g\ l^{-1}$)	0.2	13.3	0.1	0	0	13.3
Mean salinity (ppt)	18.1	3.3	21.3	26.8	14.1	11.5
Horizontal salinity gradient	12.2	25.8	28.7	15.7	21.2	32.8
Vertical salin. stratification	7.1	2.2	9.3	7.0	5.2	3.7
Mean nitrate ($\mu g\ l^{-1}$)	10.6	23.4	20.7	25.7	4.3	61.1

Discussion

As discussed in the literature review (Volume 2), salinity levels of up to 40 ppt do not affect phytoplankton productivity, rather it affects species composition. The fact that low production is often associated with high salinity conditions is a nutrient effect, i.e. lower nutrient availability usually being associated with a stronger marine influence. Nutrient availability appears to be the major freshwater-related factor controlling phytoplankton production, increased nutrient availability leading to increased production. Increased nutrient availability in estuarine waters may result from a number of processes, namely increased nutrient loading in the freshwater input, increases in the quantity of freshwater input or increases in fresh/seawater flow rates such that benthic regenerated nutrients are mixed into the euphotic zone (Flint 1985, Malone *et al.* 1988).

Consistent high nutrient loading in the freshwater input occurs in the Sundays river estuary. The consistently high nutrient levels result from agricultural fertiliser run-off from the catchment area (Emmerson 1989). In the Sundays estuary high chlorophyll-a

concentrations (defined as greater than 20 $\mu g\ l^{-1}$) are maintained if the nitrate concentration is greater than 200 $\mu g\ l^{-1}$. The retention time of the water within the estuary is also important. Hilmer and Bate (1991) found that phytoplankton blooms, formed in the upper reaches of the Sundays estuary when there was a water residence time of 7 or more neap tidal cycles and at least 3 spring tidal cycles (Mackay and Schumann 1990). The stable tidal condition allowed the phytoplankton (especially dinoflagellates) to bloom as it was present for longer than the doubling time requirements to produce the bloom. Bloom development is prevented or destroyed when rates of dilution exceed net growth rates (Peterson and Festa 1984).

Water residence times are also important in order for the phytoplankton to utilise all the nutrients. If retention time is short then there is insufficient time for the phytoplankton to utilise all the nutrients. Water residence times will depend on freshwater flow rates *per se* as well as tidal flow and the condition of the mouth. Residence time is a difficult parameter to measure. This poses problems as realistic simulation of phytoplankton populations will always be dependent upon accurate descriptions of mixing processes in estuaries (Cloern 1991a).

A similar situation where freshwater input results in consistently high nutrient levels and, therefore, large phytoplankton biomass occurs in the Great Fish estuary. Mean chlorophyll-*a* in the Great Fish estuary has been reported as 20.5 $\pm$ 31.5 $\mu g\ l^{-1}$ (Allanson and Read 1987).

In most other estuaries, increased nutrient availability results from an increase in the quantity of freshwater input. In the Great Brak and Keurbooms estuary, nutrients brought in by a freshwater pulse between August and November 1992 boosted phytoplankton biomass in the short term. Chlorophyll-*a* levels increased from < 1 $\mu g\ l^{-1}$ to 13 $\mu g\ l^{-1}$. These freshwater pulses may be very important as they serve to recharge nutrient pools (Flint 1985).

Strong estuarine phytoplankton blooms generally consist of dinoflagellates. A common bloom species in the Sundays estuary is *Katodinium rotundatum* and in the Gamtoos estuary *Prorocentrum* sp. A comparison of the phytoplankton components in 4 estuaries sampled in 1990 (Table 8) indicates that dinoflagellate numbers in the Gamtoos estuary were an order of magnitude higher than the other systems (Hilmer pers. comm., unpublished data). According to Margalef (1978), diatoms would be favoured in well-mixed, nutrient-rich waters during spring tides. Flagellates would increase in abundance when stratification sets in and nutrients became depleted. Red tide species of dinoflagellates would be favoured when a stable, stratified water column was present, but when nutrient concentrations were still high. *Katodinium rotundatum* and *Prorocentrum*

sp. are both categorized as red tide species (Bate, pers. comm.). Taxonomic phytoplankton groups appear to be spatially separated in the Gamtoos estuary. Diatoms occur in the mixed area near the mouth, *Prorocentrum* occurs in the high salinity stratified waters and picoplankton (< 2 μm) in low salinity stratified waters.

In some overseas systems zooplankton grazing affects species composition as large species such as dinoflagellates are relatively immune to grazing (Moss *et al.* 1989). In the Sundays estuary consistently high chlorophyll-a levels result in high zooplankton numbers. However the concentration of the phytoplankton bloom does not appear to be significantly reduced by zooplankton grazing (Jerling, pers. comm.).

The rules in the expert system predict whether phytoplankton will bloom and form part of the estuary after the freshwater treatment. These rules are based on the requirement for a minimum nitrate level (200 μg l^{-1}) and water retention time (3 spring tidal cycles) before phytoplankton chlorophyll-a will reach a level of 20 μg l^{-1}, i.e., apparent to the naked eye. although a knowledge of nutrient status and water retention time in an estuary are important factors used to predict phytoplankton blooms, salinity stratification is also important as it gives an indication of the stability of the water column. Knowledge of the salinity stratification can be used to predict species composition. Dinoflagellates bloom if there is a hydrodynamically stable and nutrient rich environment whereas diatom species are common in well mixed waters.

Table 8 Mean phytoplankton cell numbers (cells ml^{-1}) for the water column of four estuaries sampled in 1990 (Hilmer, pers. comm.).

	ESTUARY			
SPECIES GROUPINGS	Kromme	Gamtoos	Seekoei	Kabeljous
Diatoms	661	477	786	196
Dinoflagellates	230	3319	304	148
Picoflagellates	1899	4294	4374	2246
Euglenoids	98	198	316	156

Conclusion

Phytoplankton communities appear to be dominant in estuaries with large catchment areas characterized by adequate freshwater input. In the Sundays estuary consistent high nutrient loading in the freshwater input resulted in high phytoplankton chlorophyll-a concentrations (> 20 μg l^{-1}). In the Groot Brak and Kafferkuils estuary nutrients brought in by a freshwater pulse boosted phytoplankton biomass in the short term.

5. THE DETERMINATION AND DISTRIBUTION OF MICROBENTHIC CHLOROPHYLL-*A* IN SELECTED SOUTH CAPE ESTUARIES - A PRELIMINARY INVESTIGATION

Introduction

Studies on benthic microalgae were initiated in order to test the hypothesis that benthic microalgal productivity is increased in estuaries where a reduction in freshwater flow has limited productivity in the water column by excluding freshwater supplies of nutrients. Hilmer (1990) showed in the phytoplanktonic system of the Sundays river-estuary, that accurate chlorophyll-*a* concentrations could be estimated using routine spectrophotometric techniques. For estuarine sediments, however, where the degradation products of chlorophyll-*a* as well as other pigments are present, the estimation of chlorophyll-*a* is not a simple measurement. Hence, before the hypothesis could be tested suitable methods for measuring benthic microalgal chlorophyll-*a* had to be investigated.

This is the first study which reports on the measurement, distribution and abundance of benthic microfloral communities in small estuaries along the south and east coast of South Africa. Other studies on benthic microalgae have been conducted in Lake Sibaya (Bowen 1978), Langebaan Lagoon (Fielding *et al.*, 1988) and Swartvlei estuary (Whitfield 1989). The aim of this study was to document the amount of chlorophyll-*a* present in different locations of the longitudinal axis, as well as in different positions of the transversal axis of estuarine systems, where environmental conditions may differ. In order to achieve these aims appropriate methods to measure chlorophyll-*a* of these benthic microalgae were investigated in detail in the Swartkops estuary (Fig. 34).

Methods

The determination of optimum conditions for the extraction of benthic chlorophyll-*a*

Chlorophyll-*a* was measured because it provides an estimate of benthic microalgae biomass and can be converted into units of primary productivity. Sediment samples were collected using the coring device illustrated in Figure 35. This coring device was suitable for taking subtidal samples in water approximately 5 m deep and avoided expensive and time-consuming SCUBA diving. A sediment sample was collected with water above it. The output valve of the corer released air or water when the device was pushed into the sediment. When the corer was pulled out, the valve blocked the release mechanism thus

avoiding water flowing back past the sample. Using a rubber bung on a rod with a diameter slightly less that the core tube, the small volume of water was drained through the lower orifice off the top. The sample was then recovered from the corer by forcing the sediment core up as the bung was pushed gently upwards. The corer was constructed from perspex so that one could see and easily assess the state of the sediment sample.

One of the most important factors influencing the extraction of chlorophyll from benthic samples, is the water content. The amount of ethanol used must give a final concentration of at least 90% (Nusch 1980), hence a sampling strategy was developed to establish the minimal amount of ethanol solvent required, as well as the optimum core sizes for sand, silt and mud estuarine samples.

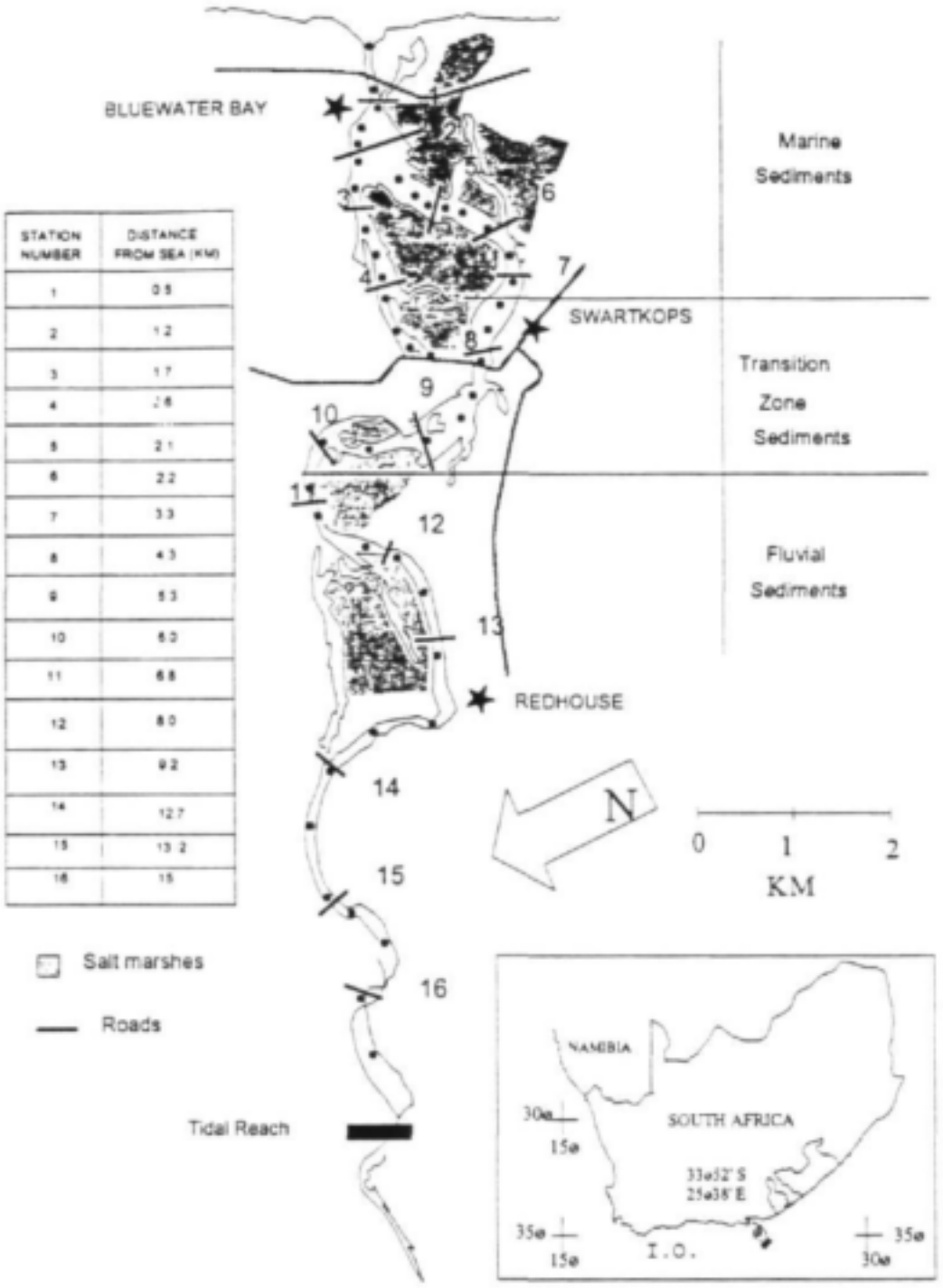
At each station in the Swartkops estuary (Fig. 34), samples were taken randomly with 12 mm internal diameter perspex cores, to a depth of 10 mm in sand, silt and mud sediments. The chlorophyll was immediately extracted for 2 hours using volumes of 10, 20, 30, 40 and 50 ml of 95% ethanol (Arvola 1981). This was done to establish the optimal ethanol volume to be added during the extraction procedure. Samples were also taken with 16, 20, 25, 30, 35, 40, 44 and 52 mm internal diameter cores, to establish the optimal core diameter. Chlorophyll concentration was calculated according to Nusch (1980) after converting the equation for use with benthic samples:

$$\text{Chl-}a\ (\text{mg m}^{-2}) = (E_{b665} - E_{a665}) \times 29.6 \times (V/A) \times 1000$$

where:

E_{b665}	=	absorbance at 665 nm before acidification.
E_{a665}	=	absorbance at 665 nm after acidification.
A	=	the area of the sample in mm^2.
V	=	the volume of ethanol used in the extraction in ml.
29.6	=	a constant calculated from the maximum acid ratio (1.7) and the specific absorption coefficient of chlorophyll in ethanol (82 $g.l^{-1}.cm^{-1}$).
1000	=	Correction factor from $\mu g.mm^2$ to mg m^{-2}

After 2 hours of extraction, the samples were centrifuged at 3000 revolutions per minute for 10 min. and the supernatant was filtered using glass-fibre filters (Schleicher & Schüll No. 6). The absorbances were measured at 665 nm in a Milton Roy Spectronic Mini 20 corrected to a Phillips PU 8700 UV\Vis according to the equation; Chl-a = (1.499)x Milton Roy Spectrophotometer reading. The data from the field spectrophotometer was also correlated to values produced by high-performance liquid chromatography (HPLC), to evaluate its suitability for field measurements.



STATION NUMBER	DISTANCE FROM SEA (KM)
1	0.5
2	1.2
3	1.7
4	2.6
5	2.1
6	2.2
7	3.3
8	4.3
9	5.3
10	6.0
11	6.8
12	8.0
13	9.2
14	12.7
15	13.2
16	15

Figure 34 Map of the Swartkops estuary. Numbered transects indicate sampling points of the present study. Dots indicate sampling points of Reddering and Esterhuysen (1981).

The monochromatic spectrophotometric method for chlorophyll-a determination involves the measurement of light absorbance by photosynthetic pigments at a given wavelength (Lorenzen 1967, Nusch 1980 and Daeman 1986). A shortcoming is that different photosynthetic pigments [for example chlorophyll-a and phaeophytin-a, Varela (1981)] absorb light in the same region of the visible spectra and, therefore, chlorophyll-a concentrations can be overestimated. Because pigments other than chlorophyll-a are present in estuarine sediments, the reliability of the spectrophotometer for chlorophyll-a determinations was investigated. Chlorophyll-a concentrations from the same sample were determined by both HPLC and spectrophotometer.

Chromatographic techniques such as liquid chromatography (HPLC) physically separate photosynthetic pigments before spectrophotometric readings are taken. Thus one can ensure that absorbance values represent the chlorophyll-a concentration of the sample being read. The HPLC determinations were done using a Spherisorb reverse phase C_{18} HPLC column attached to a Waters Lambda-Max 481 LC spectrophotometer and Waters LM-45 solvent delivery system. A 30% methanol: 70% acetone mixture was used as carrier. The system was calibrated using pure chlorophyll-a (Sigma chemicals). Concentrations were calculated with the aid of a Waters 740 data module.

In order to establish vertical profiles of chlorophyll-a distribution and the minimum sampling depth, samples were taken using 20 mm internal diameter cores every 10 mm to a depth of 50 mm in sand, silt and mud substrates.

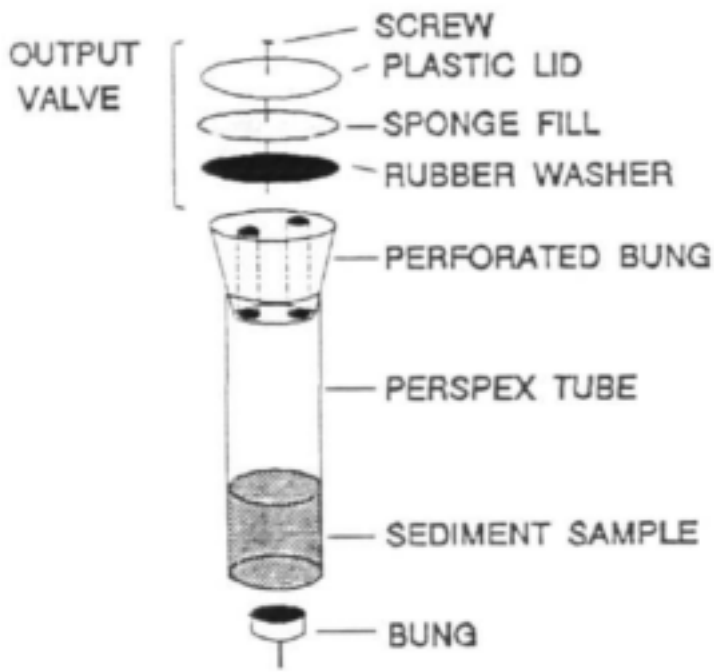


Figure 35 The coring device used to sample intertidal and subtidal estuarine sediments.

The distribution of benthic microalgal chlorophyll-*a* in the Swartkops estuary

Detailed sampling was conducted in the Swartkops estuary because of its proximity to the University of Port Elizabeth. The estuary also has a wide range of salinities and sediment types. The Swartkops estuary has a catchment area of 1365 km^2 (Reddering and Esterhuysen 1981), with a mean annual run-off of 84.42 x 10^6 m^3. The tidal reach (Fig. 34) is located 16.4 km from the sea. The estuarine water body is strongly influenced by the sea, especially in the lower reaches where salinity fluctuates between 32 ppt and 36 ppt. There is a gradual decrease in salinity up the length of the estuary (Winter 1979). The subtidal area of the estuary is 142 ha and the total intertidal area is 360 ha.

Sampling sites for our study were selected according to sediment distribution. Reddering and Esterhuysen (1981), determined the sediment composition of the Swartkops estuary from 37 stations along the main channel of the estuary. Cluster analysis of these data made it possible to reduce the number of stations from 37 to 16, with a relatively small loss of information relative to the sediment composition. At each of the 16 stations, 8 replicate samples were taken at two subtidal and two intertidal sites for benthic chlorophyll-*a* determination. Sediment samples were taken with 20 mm internal diameter corers, and immediately extracted in 30 ml of 95% ethanol for 2 hours.

It became apparent that HPLC is more accurate in determining chlorophyll-*a* in sediments than spectrophotometric methods. Problems are however raised when samples are collected where HPLC instrumentation is not available. The rate of degradation of chlorophyll-*a* was studied in order to test whether samples could be cold-stored over a number of days before HPLC measurements were made. Intertidal samples from the Swartkops estuary were used. The samples were kept in the dark at 0°C and measured by HPLC every 24 hours over a period of seven days.

Microbenthic algal chlorophyll-*a* in other estuaries of south Cape province

After establishing a reliable method for chlorophyll-*a* estimation, a number of estuaries were sampled during August 1992 along the South African coast, in order to determine the biomass and the distribution of benthic microalgae. The estuaries studied included the Berg, Kafferkuils, Gourits, Great Brak, Keurbooms, Gamtoos and Sundays. Chlorophyll-*a* was measured in the field using a portable spectrophotometer (Spectronic Mini 20). Phytoplankton chlorophyll-*a* was also measured using the method of Hilmer and Bate (1990).

Results

The determination of optimum conditions for the extraction of benthic chlorophyll-*a*

The distribution of chlorophyll-*a* in estuarine sediments is uneven. For this reason it was necessary to initially determine the minimum area as well as the optimal ethanol volume in each sample to efficiently extract benthic chlorophyll-*a*. This was determined for sand, silt and mud substrates in the Swartkops estuary. Figure 36 shows the extraction efficiency of different volumes of ethanol added to different sediment types. Chlorophyll-*a* was efficiently extracted using 10 ml of ethanol in sand, silt and mud substrates. The extraction efficiency decreased with increasing volumes of ethanol (Fig. 36). Greater quantities of ethanol might be more efficient in extracting chlorophyll-*a* from wet sediment samples but, greater quantities also yield more dilute samples which are then not efficiently measured.

Due to the spatial heterogeneity of chlorophyll distribution in estuarine sediments the optimal core size for different sediments was determined. After establishing the relationship between core area and ethanol volume for a 12 mm internal diameter core, an optimal core area was determined using different internal diameter cores but with the ethanol volume:core size ratio remaining unaltered. Sediment samples were, therefore, extracted using different core areas (ranging from 12 to 52 mm internal diameter) and different ethanol volumes (ranging from 10 to 197 ml) based on a constant of 10 ml of ethanol for a 12 mm internal diameter core.

Figure 37 shows the effect of internal diameter on the efficiency of chlorophyll-*a* extraction. Chlorophyll extraction was optimal for 20 mm internal diameter cores and 30 ml of ethanol. Thus a core of 20 mm internal diameter and 30 ml of solvent was used for all further microbenthic chlorophyll-*a* concentration determinations in this study.

Figure 38 shows the vertical profiles of chlorophyll-*a* for different types of sediment. There was no significant difference ($P < 0.05$) in chlorophyll-*a* concentrations for sand samples from the surface to 40-50 mm layer depth. For silt and mud samples there was a progressive decrease in chlorophyll-*a* concentration with depth (Fig. 38). The highest chlorophyll-*a* concentrations were obtained for the top 10 mm in both silt and mud samples. This was not the case for sand where a maximum was observed at a depth of 30-40 mm depth. However, there was no significant difference ($P < 0.05$) between chlorophyll-*a* concentrations at this depth compared to the surface layer. The silty substrates had the highest chlorophyll-*a* concentrations on the surface (ca. 76 mg m^{-2}) compared to the sandy and muddy substrates (ca. 55 mg m^{-2}).

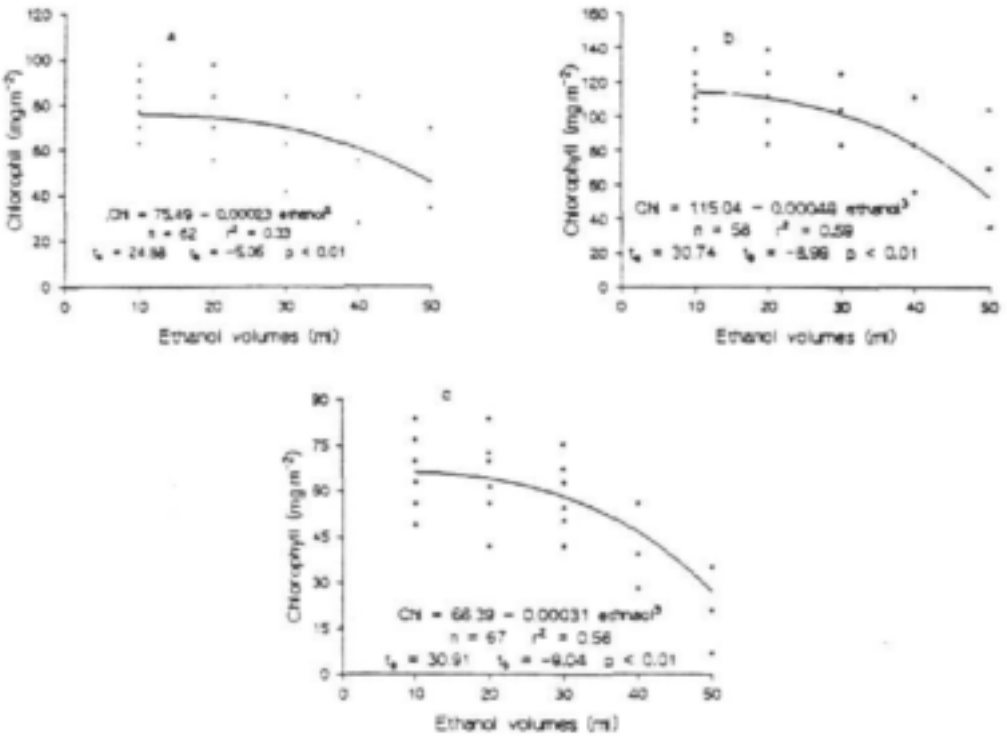


Figure 36 The effect of different volumes of ethanol for the extraction of chlorophyll-*a* using 12 mm internal diameter cores. (a) sand, (b) silt and (c) mud. (n = 14)

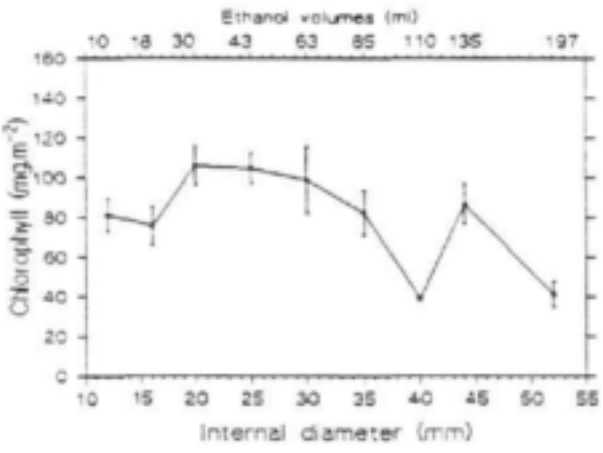


Figure 37 The effect of diameter of the sample core on the efficiency of chlorophyll-*a* extraction. (n = 5). Bars indicate 2 x SE.

The 0-10 mm layer was assumed to be a reliable sampling depth for estimating benthic microalgal chlorophyll-a in estuarine sediments and all subsequent samples were taken to a depth of 10 mm. Integrated chlorophyll-a concentrations were calculated for the entire depth profile for different sediment types (Fig. 38). Concentrations were highest in sand and decreased progressively in silt and mud sediments.

A comparison between spectrophotometer and HPLC chlorophyll-a values showed that the spectrophotometer overestimated chlorophyll-a by a mean value of 20 % for the top 10 mm of all intertidal sediments (Fig. 39). The use of the HPLC is therefore preferable compared to the spectrophotometer.

The distribution of benthic microalgal chlorophyll-a in the Swartkops estuary

After an appropriate method for measuring microbenthic chlorophyll-a concentration in sediments had been established, the spatial distribution of microbenthic algal chlorophyll-a was studied in the Swartkops estuary. Benthic microalgal biomass distribution showed a typical Gaussian distribution along the longitudinal axis of the estuary (Fig. 40).

The data show chlorophyll-a concentrations measured with both the HPLC and spectrophotometer in intertidal and subtidal sites (Fig. 40). As expected, the two-way ANOVA showed significant differences between the spectrophotometer and the HPLC techniques ($F = 61.69$, $DF = 1$, $P < 0.01$). From stations 4 to 16 there was a significant overestimation of chlorophyll-a (+45 %) by the spectrophotometer of the subtidal sites. This spectrophotometric overestimation was highest at stations 14, 15 and 16 where the visual colour of the extractions was yellow, possibly indicating a high percentage of degradation products.

Figure 40 (C) shows chlorophyll-a concentrations estimated by HPLC for subtidal and intertidal sites. The ANOVA showed a significant difference between subtidal and intertidal microbenthic chlorophyll-a values ($F = 18.91$, $DF = 1$, $P < 0.01$). From stations 1 to 9, 78 % of the subtidal chlorophyll-a values were equal to or higher than the intertidal values, from stations 10 to 16, subtidal chlorophyll-a values were in all cases lower than or equal to intertidal values.

This study has shown that the liquid chromatography technique (HPLC) is a more reliable method for measuring microbenthic algal chlorophyll-a than is the spectrophotometric technique. This poses problems when one is sampling a long distance away from the laboratory where HPLC equipment is not available. With this in mind the degradation rate of microbenthic algal chlorophyll-a was measured over a period of seven days.

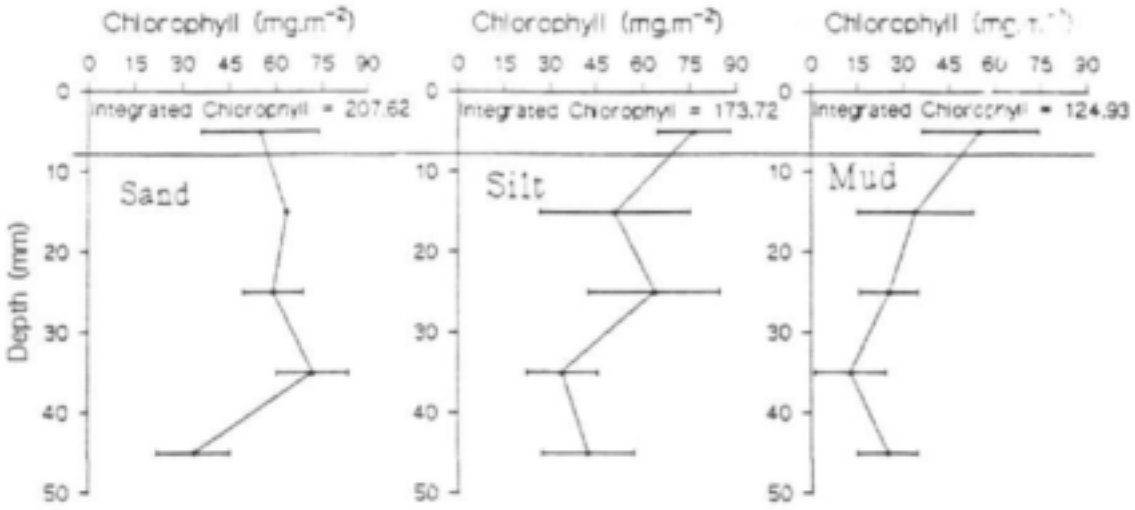


Figure 38 The content of chlorophyll-*a* at different sediment depths determined by spectrophotometer. (n = 5). Error bars = 2 x SE. Dashed lines indicate the depth of the photic zone according to Fenchel and Straarup (1971).

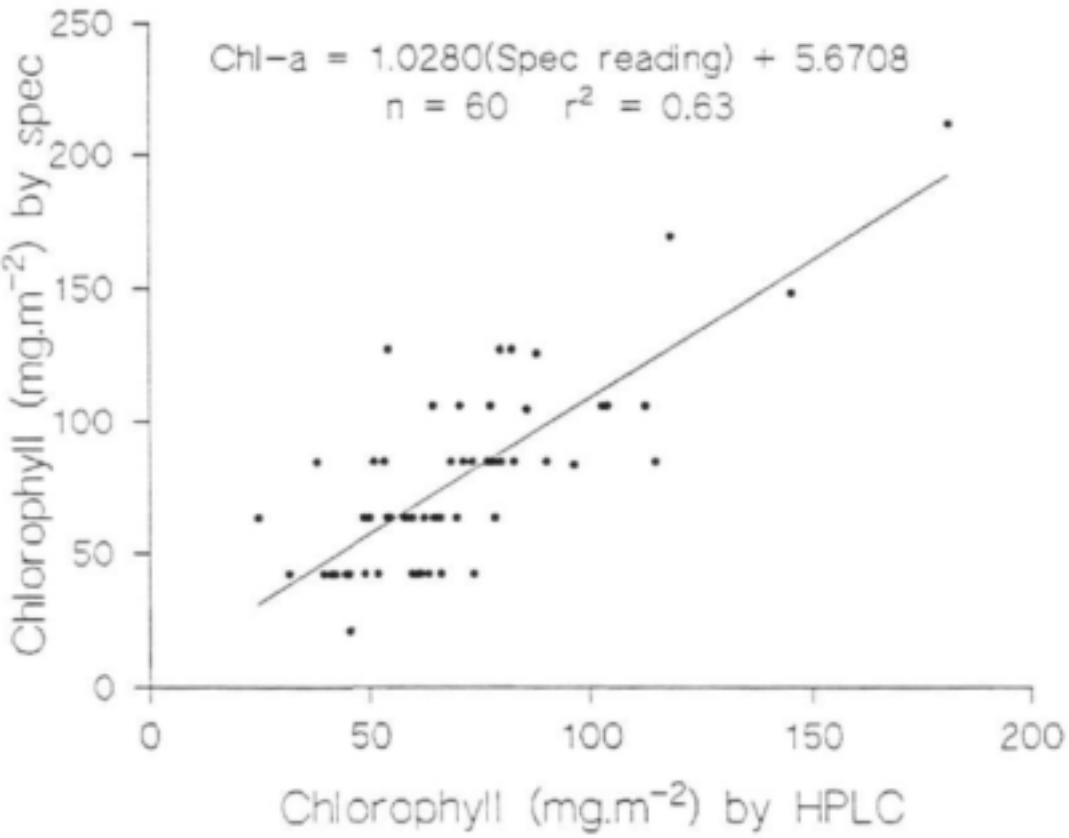


Figure 39 The relationship between chlorophyll-*a* determined with spectrophotometer and that determined with an HPLC in the top 10 mm of intertidal samples.

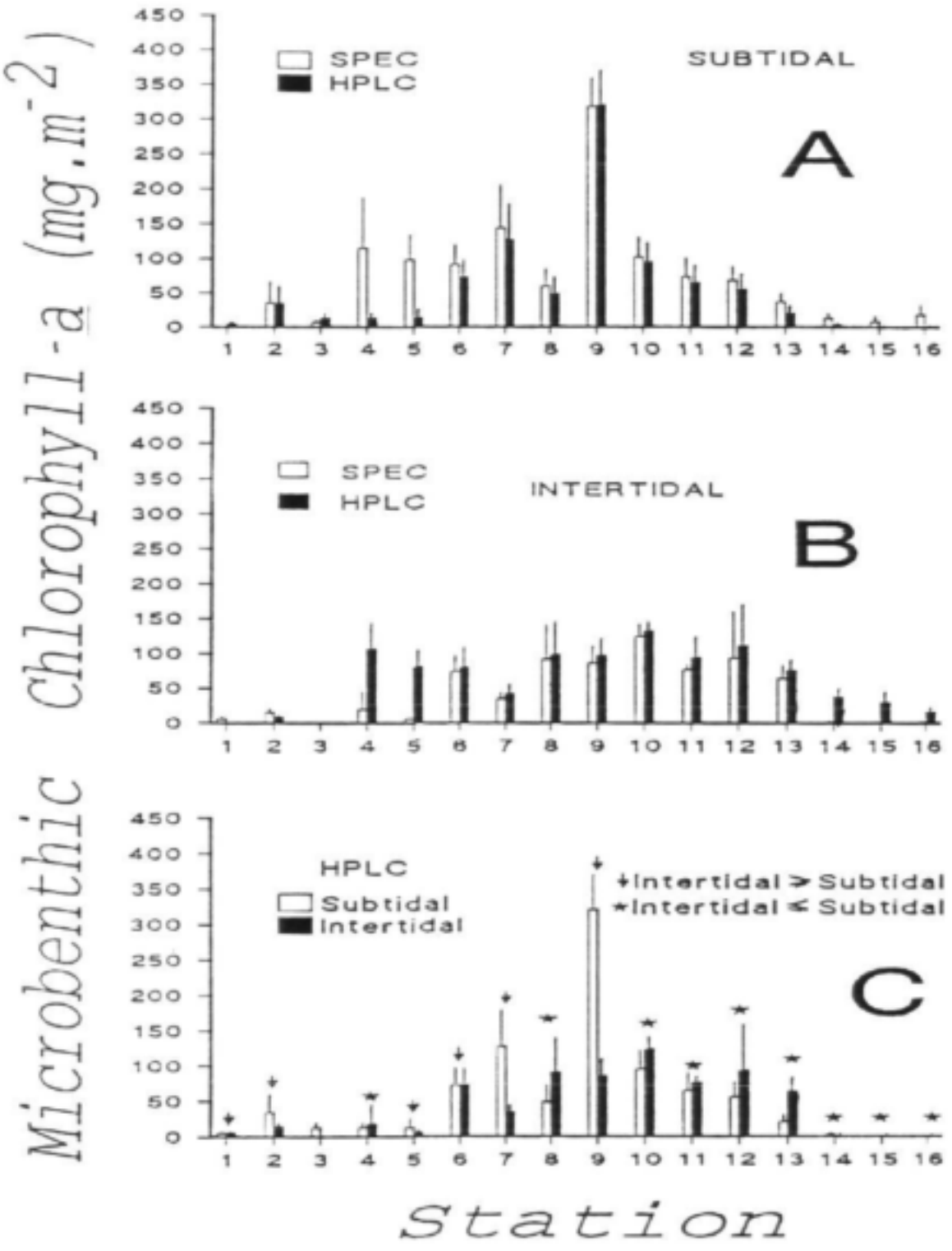


Figure 40 A comparison between Spec and HPLC for benthic Chl-a measurements, for intertidal (A) and for subtidal (B) sites. The difference between HPLC subtidal and intertidal Chl-a for the Swartkops estuary (C). Error bars = 2 x SE (n=8).

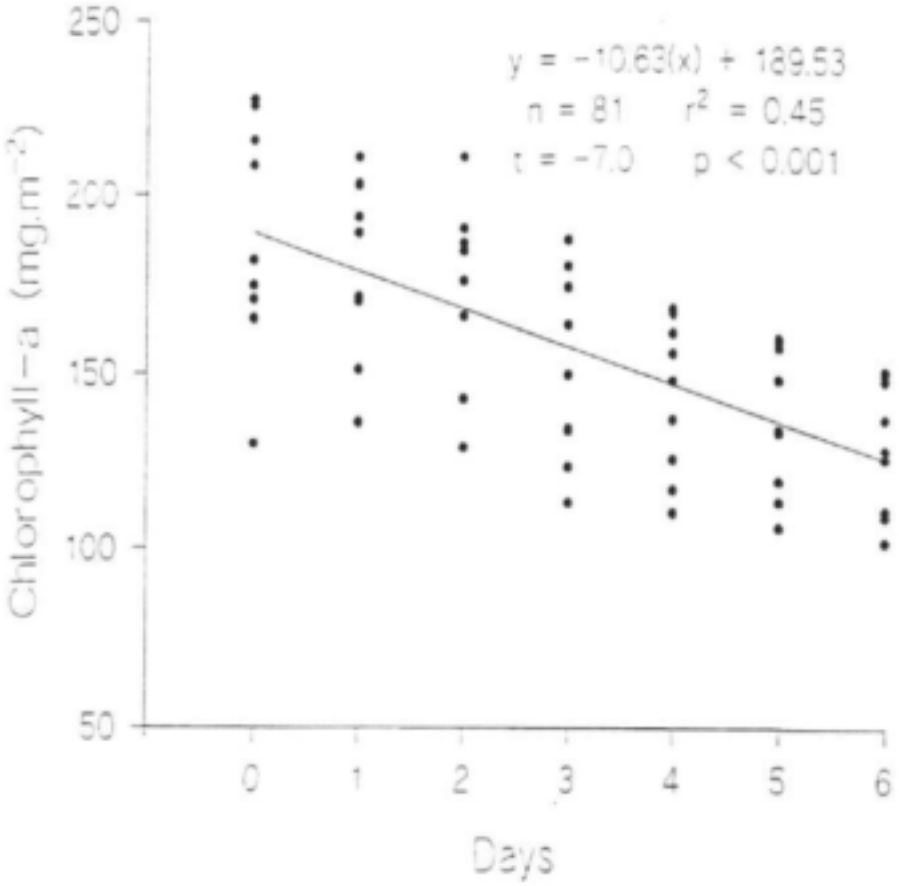


Figure 41 The degradation rate of chlorophyll-*a* over a period of one week measured by HPLC.

The degradation rate of microbenthic chlorophyll-*a* was linear over a period of 7 days (Fig. 41). In order to calculate the chlorophyll-*a* at sampling time but measured after a number of days (x), a correction equation can be applied:

Original Chl-*a* = Chl-*a* measured + (10.63 * x)

Hence, if the chlorophyll-*a* after 4 days was measured as 147.01 mg m^{-2} then the original chlorophyll-*a* at time of collection would have been:

Original Chl-*a* = 147.01 + (10.63 * 4)
= 189.53 mg m^{-2}

Thus sediment samples taken a distance away from the laboratory can be stored cold (4 °C) and later analyzed by HPLC. This technique would allow reliable determinations of benthic microalgal chlorophyll-*a* concentrations for different estuaries along the South African coast where HPLC equipment is not available.

Microbenthic algal chlorophyll-a in other estuaries of south Cape province

Table 9 shows microbenthic algal chlorophyll-a concentrations obtained in various estuaries from the south Cape. The maximum mean chlorophyll-a concentration for intertidal sites was obtained in the Sundays estuary (197.02 mg m^{-2}) while for subtidal sites the maximum mean chlorophyll-a concentration was obtained in the Kafferkuils estuary (204.8 mg m^{-2}).

Phytoplankton chlorophyll-a concentrations for the same estuaries are shown in Table 10. The maximum mean phytoplankton chlorophyll-a concentration (37.71 $\mu g\ l^{-1}$) was obtained in the upper reaches of the Sundays estuary (station 4). The Keurbooms, Kafferkuils and Gourits estuaries, and stations 2; 3 and 4 of the Berg estuary all had chlorophyll-a concentrations lower than 0.1 $\mu g\ l^{-1}$.

Mean chlorophyll-a values were obtained for the sediment and the water column of each estuary. In all cases, microbenthic chlorophyll-a was higher than phytoplankton chlorophyll-a. The mean phytoplankton chlorophyll-a concentration for all systems was 7.22 $\pm$ 2.67 $\mu g\ l^{-1}$ (or 7.22 mg m^{-3}), with 133.69 $\pm$ 24.46 mg m^{-2} (or 13.37 mg m^{-3}) and 105.35 $\pm$ 19.3 mg m^{-2} (or 10.57 mg m^{-3}) being respectively the mean intertidal and subtidal values of microbenthic chlorophyll-a.

Table 9 Microbenthic algal chlorophyll-a values obtained in various estuaries of the south Cape. Max = maximum, Min = minimum and $\pm$ SD, standard deviation. Estuaries are ranked by geographical position from west to east. (n = 4).

ESTUARY	INTERTIDAL			SUBTIDAL		
	Max	Min	Mean($\pm$SD)	Max	Min	Mean($\pm$SD)
BERG	95.33	0	55.6 (20.0)	42.37	0	26.48(9.8)
KAFFERKUILS	158.9	95.3	115.6 (14.6)	275.4	148.3	204.8 (34.7)
GOURITS	183.3	105.9	137.7 (26.6)	257.4	63.56	169.5 (44.0)
GREAT BRAK	296.6	84.7	163.1 (75.7)	296.6	105.9	166.8 (44.2)
KEURBOOMS	190.7	105.9	137.7 (26.6)	257.4	63.56	169.5 (44.0)
GAMTOOS	201.3	60.26	105.9 (50.8)	275.4	63.56	169.5 (44.0)
SUNDAYS	254.2	159.6	197.1 (31.3)	233.1	104.9	135.2 (20.5)

Table 10 Phytoplankton chlorophyll-a concentrations obtained in various estuaries of the south Cape. SE = standard error. Estuaries are ranked by geographical position (n = 4).

PHYTOPLANKTON Chlorophyll-*a* ($\mu g\ l^{-1}$)		
ESTUARY	Station	Chlorophyll-*a* ($\pm$SE)
BERG	1	19.97 (2.86)
	2	0 (0)
	3	0 (0)
	4	0 (0)
KAFFERKUILS	1	0.02 (0.008)
	2	0.02 (0.01)
	3	0.03 (0.003)
	4	0.05 (0.02)
GOURITS	1	0 (0)
	2	0.03 (0.002)
	3	0.03 (0.004)
	4	0.05 (0.003)
	5	0.21 (0.15)
GREAT BRAK	1	0.15 (0.02)
	2	0.14 (0.06)
	3	0.34 (0.23)
	4	0.13 (0.05)
	5	0.14 (0.03)
KEURBOOMS	1	0.02 (0.002)
	2	0.04 (0.01)
	3	0.04 (0.007)
	4	0.04 (0.003)
	4	0.04 (0.002)
GAMTOOS	1	1.53 (0.74)
	2	7.76 (3.0)
	3	7.76 (3.52)
	4	13.13 (3.3)
	5	2.05 (0.41)
SUNDAYS	1	18.86 (6.9)
	2	21.07 (4.9)
	3	29.95 (8.7)
	4	37.71 (10.5)
	5	35.6 (3.6)

Chlorophyll-*a* contents were calculated for both the water column and sediments for whole estuaries (Table 11). Chlorophyll-*a* biomass is expressed in terms of kg chl-*a* per estuary based on the area (ha) of the estuary. Chlorophyll-*a* concentrations were calculated using the following equations for phytoplankton and benthic microalgae respectively:

$$\sum \left\{\left(\int \text{Chl-a} \times 10000\right) \times \frac{\text{Area}}{N}\right\}_1 + \ldots\ldots + \left\{\left(\int \text{Chl-a} \times 10000\right) \times \frac{\text{Area}}{N}\right\}_n$$

$$\sum \left[\left[\left(\frac{\text{Chl-a}_s + \text{Chl-a}_i}{2}\right) \times 10000\right) \times \frac{\text{Area}}{N}\right]_1 + \ldots\ldots + \left[\left[\left(\frac{\text{Chl-a}_s + \text{Chl-a}_i}{2}\right] \times 10000\right) \times \frac{\text{Area}}{N}\right]_n$$

Where:

$\int$**Chl-a** is the phytoplankton integrated chlorophyll-*a* concentration in depth in mg m^{-3}.
Chl-a$_s$ is subtidal microbenthic chlorophyll-*a* in mg m^{-3}
Chl-a$_i$ is intertidal microbenthic chlorophyll-*a* in mg m^{-3}
N is the number of stations taken along the longitudinal axis of the river-estuary.
10000 is the correction factor from metres to hectares.
Area is the area of the estuary in hectares.

Higher chlorophyll-*a* concentrations were observed in the sediment than in the water column for all systems except for Sundays and Gamtoos estuaries (Table 11). The microbenthic component was two and three orders of magnitude higher than phytoplankton, while phytoplankton was higher than benthic microflora in the same order of magnitude.

Table 11 Phytoplankton and microbenthic chlorophyll-*a* biomass calculated for the whole estuary in kg.estuary^{-1}. Estuaries are ranked by geographical position from west to east.

ESTUARY	PHYTOPLANKTON	MICROBENTHIC
KAFFERKUILS	0.07	23
GOURITS	0.04	16
GREAT BRAK	0.4	62
KEURBOOMS	0.06	20
GAMTOOS	17	14
SUNDAYS	86	14

Discussion

Appropriate methods for measurement of benthic chlorophyll-*a* were investigated in detail in the Swartkops estuary. When sediment samples are taken with different internal core diameters a suitable volume of ethanol must be added. This study showed that 10 ml was the optimal volume of ethanol for a sample with an internal core diameter of 12 mm. The highest extraction efficiency was obtained with 20 mm cores using 30 ml ethanol. In samples smaller than 20 mm internal diameter, chlorophyll appears to be underestimated due to sediment disturbance. Ethanol is the preferred solvent because it is relatively cheap and is far less toxic than the alternatives acetone or methanol.

Since the relationship between the depth of the photic zone and sediment type changes for different sediment types (Fenchel and Straarup 1971), the vertical distribution of chlorophyll-*a* was determined in sand, silt and mud substrates. The results indicated that the surface layer down to 10 mm depth included most of the chlorophyll-*a* concentrations for all sediment types. This agrees with de Jonge (1980), Shaffer and Onuff (1983), Varela and Penas (1985) and Lukatelich and McComb (1986) who also sampled the 0-10 mm layer of estuarine sediments.

A comparison between spectrophotometric and HPLC techniques showed that the spectrophotometer overestimated the content of chlorophyll-*a* on average by 20 %. This agrees with Daemen's (1986) work, who reported that a spectrophotometric overestimation in chlorophyll-*a* concentration existed but never exceeded 30%. This indicates the necessity for information about the pigment composition. Chlorophyll, and the degradation products of chlorophyll, absorb or fluoresce, in the same range of the visible spectra. Therefore in systems with high concentrations of degradation products, traditional spectro-fluorometric techniques will be unreliable. The use of chromatographic techniques is therefore essential to evaluate true chlorophyll-*a* without interference from breakdown products in sediments.

A major problem in this study was to develop a reliable method that involved the chlorophyll-*a* determination by HPLC for study areas situated a long way from the laboratory. It is not possible to take an HPLC into the field and this was essentially a field study. The degradation rate of microbenthic chlorophyll-*a* under refrigerated (4 °C) conditions was shown to be linear over a period of 7 days (Fig. 41). This is important, because by calculating the value of the degradation slope, one can, by multiplying this factor and the number of days that the degradation had progressed, arrive at a factor

which allows one to calculate the initial chlorophyll-*a* concentrations for up to 7 days after the sampling exercise. The value of the degradation slope of microbenthic chlorophyll-*a* was 10.63 (Fig. 41). Hence, by multiplying 10.63 by the number of days since sampling, and then adding this factor to the actual measured chlorophyll-*a*, the correct original value can be obtained.

Using this correction factor, samples taken a long distance away from the laboratory can be brought back and subsequently analyzed. The method has obvious faults but was the best solution found to determine the active microphytomass for estuarine ecosystems along the coast of South Africa.

In terms of microbenthic chlorophyll-*a*, the Swartkops estuary can be divided into three sections (a), (b) and (c) (Fig. 42). Both sections (a) and (c) appear to have low benthic biomass (Fig. 40). The estuary has a permanent open-mouth and in section (a), diurnal tidal currents cause eddies, turbulence, residual currents and wind-action-generated waves which cause microalgal resuspension from the sediment. This reduces benthic microalgae colonization and is the most likely reason for low chlorophyll-*a* values recorded in the mouth area.

Chlorophyll-*a* concentrations were higher in the middle reaches (b) of the estuary compared to the mouth and upper reaches. In this area sediment deposition is greater than resuspension (Postma 1967, Delgado *et al.* 1991) allowing microalgal colonization. Diatoms form films and produce mucilage threads (Holland *et al.* 1974, Grant *et al.* 1986) which stabilize sediments. This is an important survival strategy under dynamic estuarine conditions.

The low benthic chlorophyll-*a* concentrations in section (c), the upper reaches of the estuary, may relate to flow velocities and the time available for growth. The combination of low density of cells attached to particles (Jonge 1985) and the relatively high percentages of fine sediments, is responsible for the low sediment stability in the upper reaches of the estuary. The resuspension of sediment in this section of the estuary is mainly caused by biophysical processes (Margalef 1983) such as bioturbation and not by physical processes of the water column, and therefore the sediment becomes anoxic, due to the lack of mixing processes in the water column.

The HPLC chlorophyll values for section (a) were higher than the spectrophotometer readings, because the HPLC is more sensitive and can read low values. The presence of

chloroprhyll-a measured by spectrophotometer in section (c), does not necessarily imply benthic microfloral presence in the sediment column. The extracted chlorophyll samples were generally yellow in colour and this was possibly due to the high concentrations of detrital compounds (Delgado *et al.* 1991). In this area the allochthonous contribution represents an important fraction of the total budget of the present biomass and the relative amount of chlorophyll-a decreases.

In the lower reaches of the estuary (stations 1 to 7) subtidal chlorophyll-a values were higher than or equal to intertidal values. From stations 10 to 16, subtidal chlorophyll-a values were lower than or equal to intertidal values. Stations 8 and 9 represent a transition zone. Thus, by comparison of subtidal and intertidal chlorophyll-a levels, the estuary can be divided into sections I (stations 1 to 7) and II (stations 10 to 16, Fig. 42).

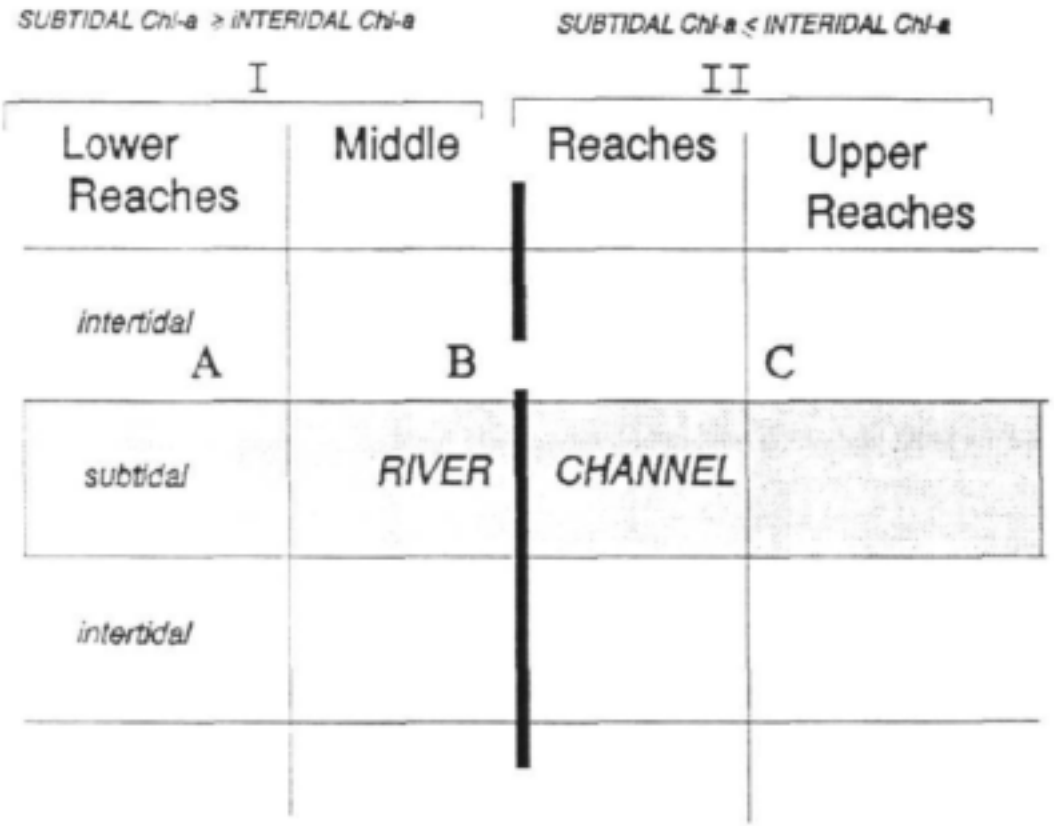


Figure 42 Diagram to depict the chlorophyll-a distribution in the Swartkops estuary. Different sections showed significant difference ($p < 0.01$) in chlorophyll-a concentrations.

The low intertidal chlorophyll-*a* values in the lower reaches of the estuary could be due to tidal fluctuations which are higher in the lower reaches of the estuary. Desiccation, emersion and residence times for benthic microalgae are consequently more variable (McIntire 1978, Davis and McIntire 1983). In the upper reaches of the estuary, tidal fluctuations affecting the intertidal sediments are reduced and the subtidal-environmental conditions (eg. anoxic sediments) are not favourable for benthic microalgae colonization. Therefore intertidal chlorophyll-*a* concentrations are higher than or equal to subtidal chlorophyll-*a* concentrations.

McIntire and Overton (1971) and Main and McIntire (1974), found a positive correlation between salinity and benthic diatom biomass. Moore and McIntire (1977) found a distributional continuum in Yaquina estuary (Oregon, U.S.A.) with a pronounced discontinuum in benthic microalgae where the salinity fluctuated around a mean value of 5 ppt. The results in this report (Fig. 40) support the idea that there is a correlation between salinity and microphytobenthic biomass. Chlorophyll-*a* values were higher in the brackish middle reaches of the estuary. Towards the mouth of the estuary, the physical perturbation of the sediment surface by physical processes of the water column, becomes hierarchically more "important" than salinity, as a factor controlling benthic microalgae biomass. Thus, chlorophyll-*a* concentration were low in stations 1; 2 and 3 (Fig. 40).

Because the degradation products of chlorophyll-*a* represent an important fraction of the total pigments of estuarine sediments, traditional spectrophotometric (or fluorometric) techniques are unsuitable for measuring benthic floral pigments. The HPLC is the best method for measuring chlorophyll-*a* in sediments, since physical separation of pigments takes place before the absorbances (or fluorescence) are read.

The data from this study are comparable to other studies done in South Africa on benthic microalgae. The mean benthic chlorophyll-*a* for all the estuaries studied was estimated to be 119.14 mg m^{-2}. In the Swartvlei estuary benthic chlorophyll-*a* concentrations were less than 80 mg m^{-2} (Whitfield 1989), and less than 100 mg m^{-2} in Langebaan lagoon (Fielding *et al.* 1988). Microbenthic chlorophyll-*a* concentrations obtained in this study were in the same range as those obtained by Cadee and Hegeman (1974) for the western Dutch Wadden sea. They measured a maximum chlorophyll-*a* concentration of 435 mg m^{-2}, which is one of the highest values reported in the literature. The highest microbenthic chlorophyll-*a* value reported in this study (423.7 mg m^{-2}) was found in the Great Brak estuary, at station 4 which was located 5.3 km from the sea.

Chlorophyll-*a* contents calculated for the water column and sediments for the whole estuary showed that in most estuaries the microbenthic component was two or three orders of magnitude higher than the phytoplankton. The exception were Sundays and Gamtoos estuary. These data show that the benthic component would play an important role in energy fixation and the food web of these estuaries.

6. MICROALGAL IDENTIFICATION

Introduction

Phytoplankton and benthic microalgal species have been sampled in a number of estuaries between the Berg and Great Fish estuary. An inverted light microscope was initially used for identification and cell counts. However, in most cases it was difficult to identify specimens to species level and scanning electron microscope (S.E.M.) techniques were then used.

Methods

Samples were taken at 4 stations in each estuary from the mouth to the upper reaches or freshwater source. Phytoplankton samples were taken from a boat along the centre of the estuary, at different depths. Benthic microalgae samples were taken using a corer, in the intertidal and subtidal zones. The first 2 cm sediment layer was sampled. Field work consisted of field microscope observations, field microscope photographs of live specimens and the preparation of the microalgal cultures. A few drops of neutralized formalin (20%) was added to one sample to be used for the quantitative study (i.e. cell counts). Another sample was taken for culturing in Provasoli's ES medium. In the laboratory, cultures of isolated cells were prepared. Each isolated species was cultured in full-strength Provasoli's ES medium (Pravasoli 1968) which was adjusted to the same salinity as was measured in the field. The larger cells did not grow in pure culture and were therefore cultivated in mixed cultures. For the pure cultures, a single cell was picked up using a micropipette and mixed with the culture medium. The culture was allowed to grow for 2 to 3 weeks, before Provasoli's ES medium was added. For the mixed cultures, 2 to 3 ml of sample was mixed with the culture medium and allowed to grow for 3 to 4 weeks.

The benthic samples were prepared for quantitative analysis by mixing 5 g sediment with 20 ml of autoclaved seawater or elution mixture (25 % lactic acid). This was shaken (20 X clockwise and 20 X anti-clockwise and the liquid poured off. The procedure was repeated 3 times in order to separate the cells from the sediment. Samples were examined with a Zeiss IM 35 inverted light microscope ICM 405 and with a Scanning Electron Microscope ISI ABT DS-130S (1st stage = resolution 30°A, 2nd stage = resolution 40°A). It was not possible to identify to species level using light microscopy. However light microscopy was necessary for quantitative analysis. Stubs were prepared for electron microscopy by boiling a concentrated microalgal sample in a solution of 50% nitric acid for 15 min. The frustules were then washed several times with distilled water to remove the acid. The solution was filtered under vacuum through Nucleopore membrane

filter paper, dried and mounted on a S.E.M. metal stub. Stubs were then sputter-coated with gold palladium and kept in a desiccator.

Results

Microalgae were divided into the following groups: diatoms, dinoflagellates, picoflagellates, euglenoids, coccolithophorids, green and blue-green algae. Species identification concentrated on diatoms which were the dominant fraction of the benthic community.

Species lists were compiled for:

a) Phytoplankton (diatom) species found in Cape estuaries sampled,
b) Benthic microalgae (diatom) species found in Cape estuaries sampled,
c) Species associated with marine (20-35 ppt), brackish (5-20 ppt) and freshwater (< 5 ppt) regions of the estuary.

These lists are in the appendix as Table 1, 2 and 3. This was a qualitative analysis of species found during various sampling trips (August 1992, November 1992, March 1993 and July 1993). Light and scanning electron micrographs are given for a few species.

Although the literature indicates that diatoms are the most important component of the pelagic estuarine flora, our studies have shown that flagellates are by far the most important. Including flagellates with benthic diatoms in the taxonomic survey was too large for us to handle. For this reason and because of the much larger contribution of benthic microalgae to total productivity in most Cape estuaries, we have concentrated on diatoms. A study of the flagellate population urgently requires examination.

Discussion

In order to understand and manage estuaries information on benthic microalgae and phytoplankton ecology is required. Without the name of the species the data cannot be published. Identification down to species level requires the use of a Scanning Electron Microscope. With the help of Dr G. Boalch (Plymouth Marine Laboratory, Great Britain), we have started to identify some species. Previously very little information was available on phytoplankton and benthic microalgae communities in South African estuaries. Now preliminary species lists are available for some Cape estuaries

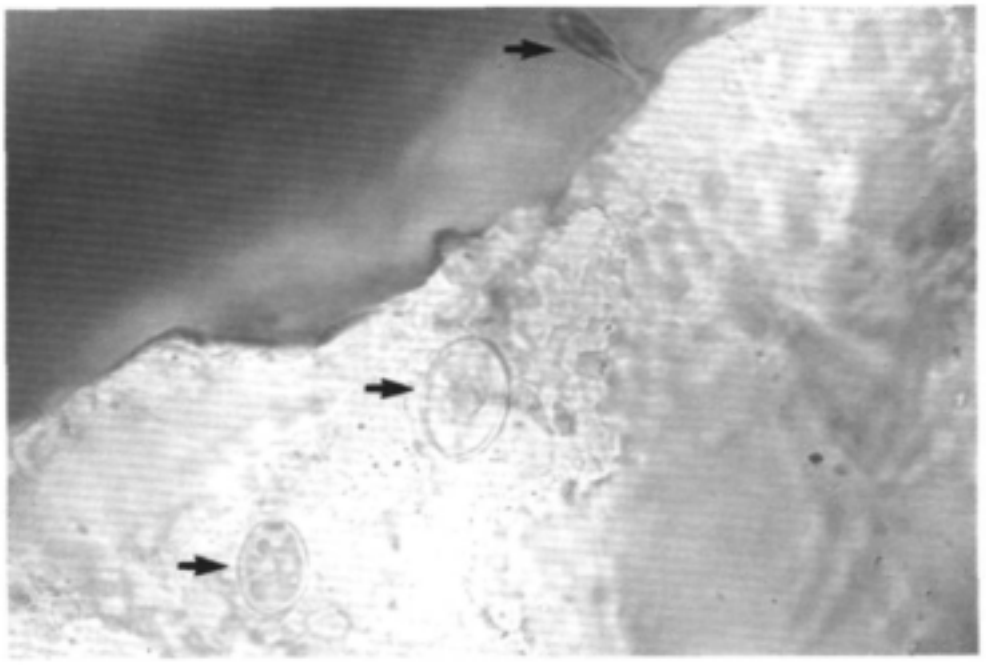


Plate 10 Light micrograph of *Cocconeis* sp.1. attached to a sand grain. *Cocconeis* spp were found to be predominantly benthic and occurred over a wide salinity range from freshwater to marine conditions. (Scale: 4.9 cm = 50 μm).

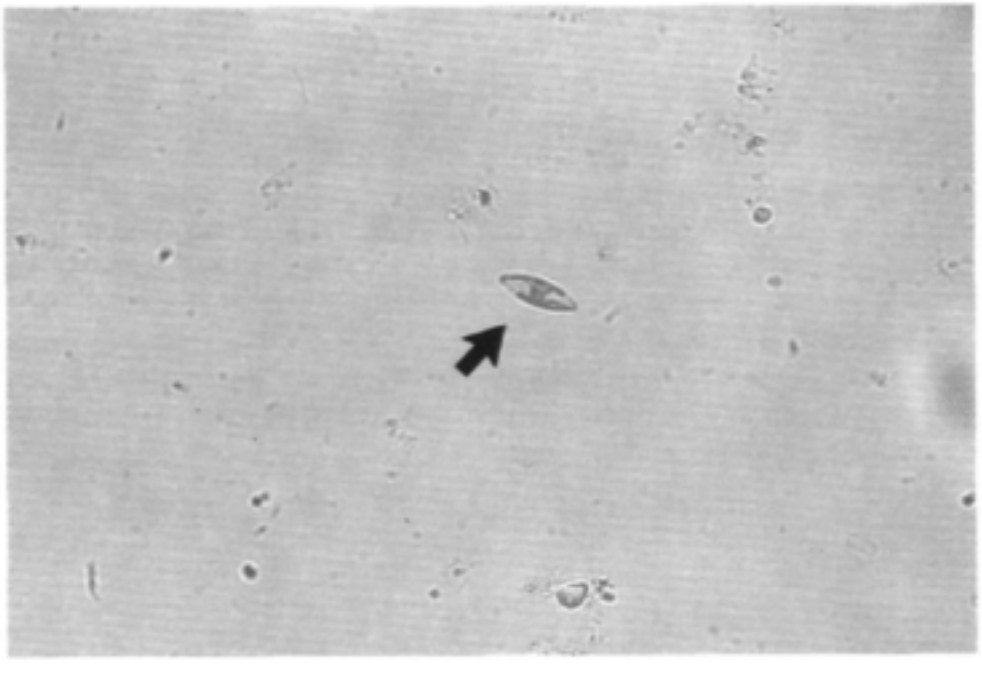


Plate 11 Light micrograph of *Navicula* sp.9. This species was named "tiny *Navicula*" because of its small size (Scale: 4.9 cm = 50 μm). It is planktonic and occurred in most estuaries sampled (Berg, Palmiet, Kafferkuils, Gouritz, Great Brak, Keurbooms, Gamtoos and Sundays).

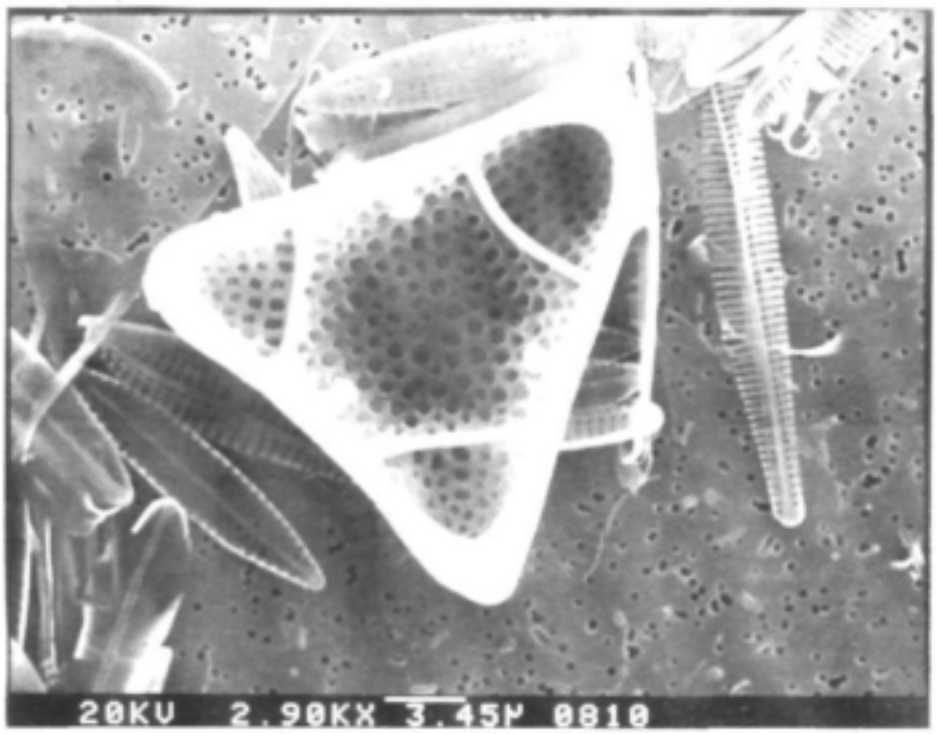


Plate 12 SEM of *Biddulphia alternans* which occurred at brackish sites in the Kafferkuils, Keurbooms and Gamtoos estuaries.

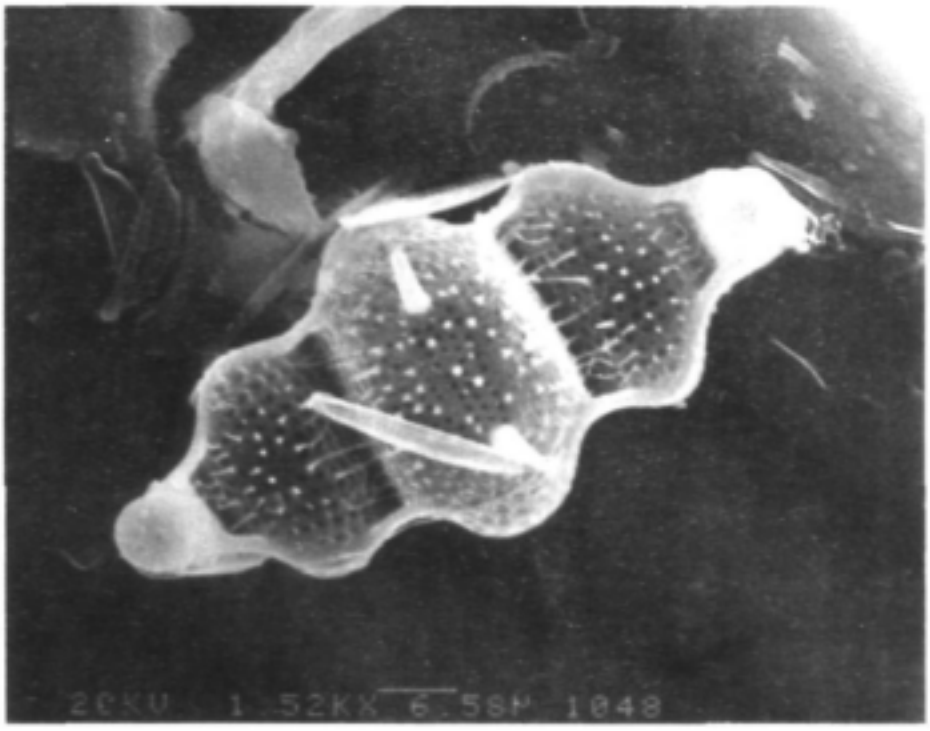


Plate 13 SEM of *Biddulphia tuomeyi* which occurred at brackish sites in the Kafferkuils and Keurbooms estuaries.

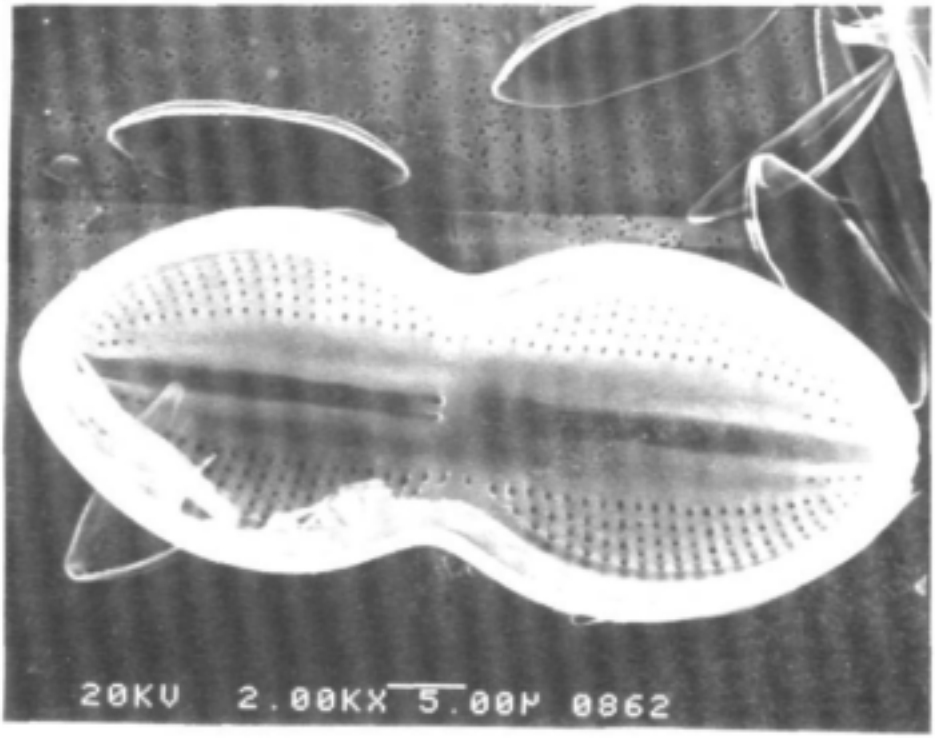


Plate 14 SEM of *Diploneis didyma* which was only found in the Gamtoos estuary.

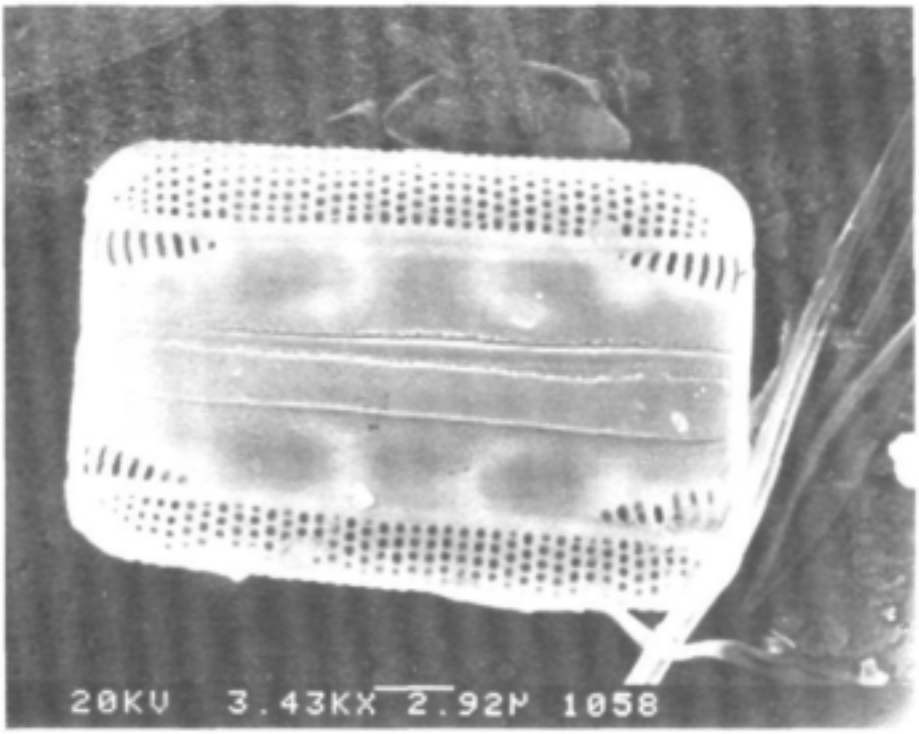


Plate 15 SEM of *Grammatophora* sp. which was only found in the Keurbooms estuary.

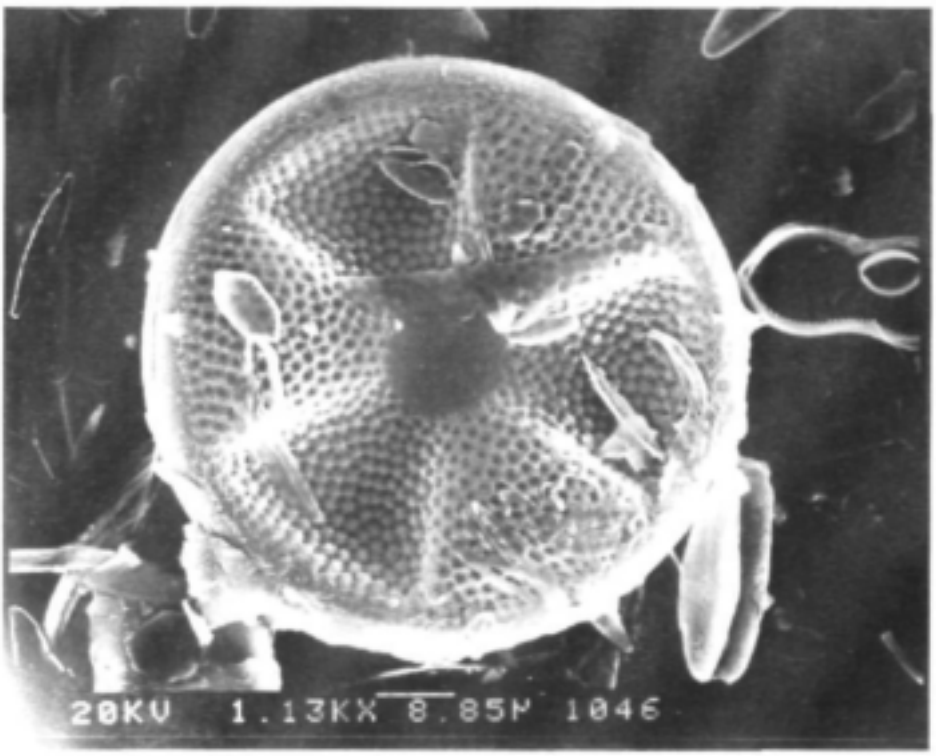


Plate 16 SEM of *Actinoptychus undulatus*, a phytoplankton species found at marine sites in the Kafferkuils, Gouritz and Keurbooms estuaries.

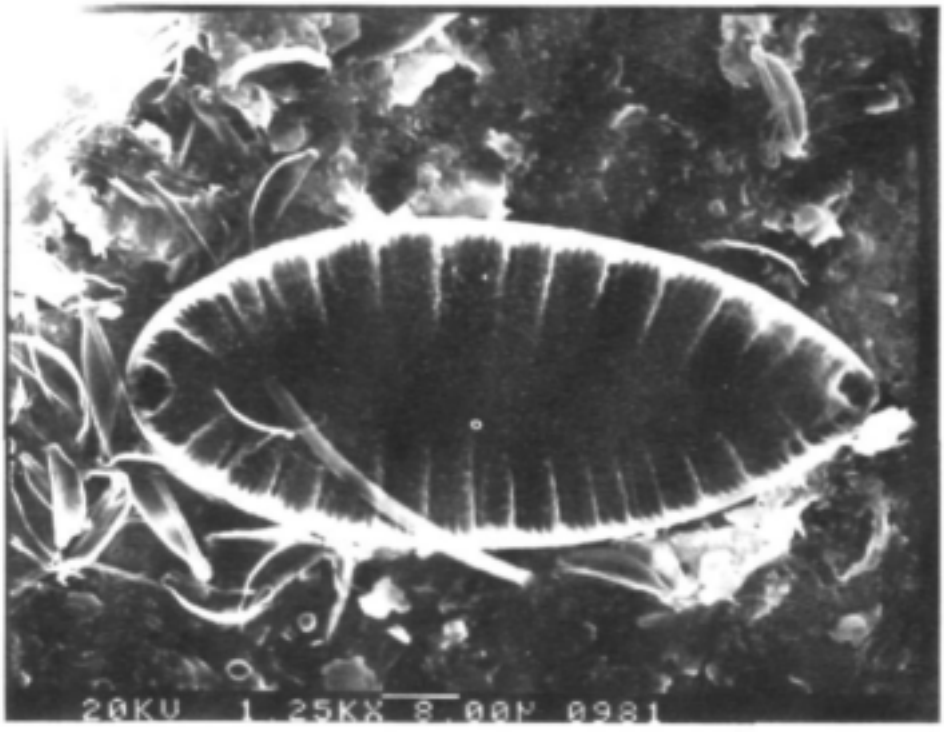


Plate 17 SEM of *Surirella* sp., a phytoplankton species found at freshwater sites in the Berg, Kafferkuils, Groot Brak and Keurbooms estuaries.

7. A DECISION SUPPORT SYSTEM TO AID IN THE MANAGEMENT OF THE FRESHWATER REQUIREMENTS OF ESTUARINE PLANTS

Introduction

The first prototype expert system (EDSSys) was developed early in this research programme to aid water management decisions relating to the response of estuarine plants to different freshwater input scenarios. While the ultimate aim of the expert system was to aid water managers, the early data used established the hypotheses subsequently tested in the detailed research.

The expert system was developed for water managers who have little knowledge of estuarine plants. In particular, the system was designed to answer questions on how changed inflow conditions (addition or abstraction of freshwater) would affect estuarine plants. The purpose of the expert system is to predict whether macrophytes will die, show reduced growth or be unaffected by a manipulation of freshwater inflow. It also aims to predict whether phytoplankton will bloom and form a feature of the estuary after the manipulation. The expert system predicts plant response to changes in a number of physical factors namely: salinity, water level, current velocity and water turbidity. Nitrate concentrations are also considered for the phytoplankton component. The expert system is designed to predict plant responses to physical factors directly affected by freshwater input, it does not focus on plant responses to water quality (i.e. the effect of industrial pollutants on the plants).

The second prototype expert system, PEDSSys (Plant estuarine decision support system) was developed by Dr M. Brinck of MarBri Strategies. PEDSSYs was developed using the expert system shell dmX in a Windows environment. PEDSSys uses bilinear logic instead of "if-then" rules. The results for the estuarine macrophytes are based on growth rate adjustment with a score range of -10 to +10. The score for phytoplankton and benthic microalgae is based on biomass and the score range is 0 to 10. Seasonality with regard to plant responses has been included in PEDSSys. The response of benthic microalgae to freshwater input has also been included and phytoplankton have been separated into diatom and dinoflagellate communities.

Why use an expert system ?

Expert systems are an offshoot of a more general study known as artificial intelligence (AI). In the simplest sense AI is the study of computer programs that exhibit human-like intelligence (Durkin 1990). Expert systems can provide ecologists with valuable tools for

managing data and interacting with other fields of expertise. When working with ecological problems, the domain under consideration is usually very broad (Noble 1987). Thus while expert systems provide advice on small sections of wider problems, the long term goal is ultimately to link individual expert systems of different domains to cover the whole.

Expert system structure

EDSSys (Estuarine Decision Support system)

The first prototype expert system, EDSSys was developed by Ms C. Cronin as an honours project in the Computer Science department at the University of Port Elizabeth. The expert system shell, Personal Consultant Plus (PCPlus) was used. The three main components of an expert system are (i) a knowledge base, (ii) an inference engine and (iii) a user interface (Graham and Llewelyn-Jones 1988). The user interface provides a method for the user to communicate with the system and for the system to communicate back to the user. The expert system asks questions requiring answers that can be given using a natural-language style (Durkin 1990).

The inference engine or expert system shell does the actual work of the expert system. It takes the various inputs, considers existing knowledge and adds information to the knowledge base until it can reach a conclusion. Any data that the inference engine requires to perform its task of evaluating goals is obtained either from the existing knowledge base or during interaction with the user (Cronin 1991).

PCPlus is an expert system shell that uses both a frame-based and a rule-based representation of information in the knowledge base. A rule-based representation method is appropriate for this application, since the information is easily transferred into rules. The knowledge is represented as "if-then" rules. For example:

IF:: The submerged plant *Ruppia cirrhosa* is exposed for greater than three months.
THEN: The plants will die-back.

A frame-based approach is also appropriate since in estuarine plants the knowledge breaks down naturally into different modules. The knowledge within a given frame is in some way inter-related. Five modules/frames exist within EDSSys. They are: estuary, submerged macrophytes, emergent macrophytes, salt marsh macrophytes and phytoplankton (Fig. 43).

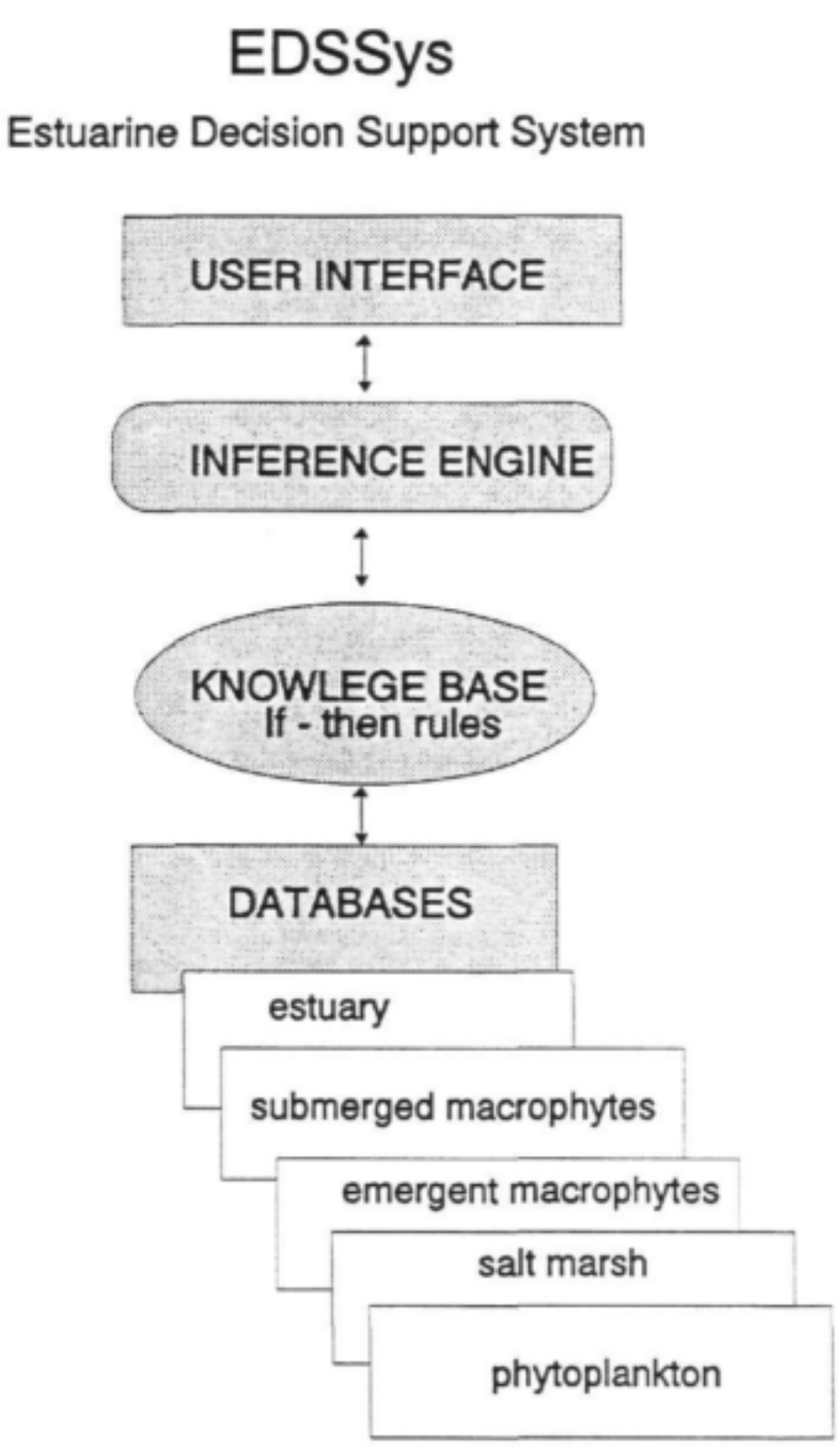


Figure 43 Structure of the expert system EDSSys

The estuary module deals with the upper level of data required for EDSSys to operate. Its function is mostly that of data gathering. The data it gathers are:

-The treatment applied to the estuary,
-the duration of the treatment,
-the plant to be considered and
-whether the plant is present in the mouth,
head and/or middle of the estuary.

The goal of the macrophyte modules is to determine whether the macrophytes under consideration will survive unaffected, show reduced growth or die in three regions of the estuary. The expert system therefore concentrates on the negative effect on plant growth, with the focus on identifying management options which cause the present conditions to deteriorate. Management to improve the present situation is very important but would require a different approach. The goal of the phytoplankton frame is to establish whether phytoplankton will bloom and occur in the estuary after the treatment.

PCPlus, the expert system shell used is easy to debug since its logic is easy to follow. The debugging task is made simpler by the fact that a trace facility exists to enable the developer to determine the logic the system is following. Besides providing results or conclusions, the expert system can also explain how it arrived at the result. This is important as it provides a justification of the results for the user (Durkin 1990).

PEDSSys (Plant Estuarine Decision Support system)

Towards the end of 1993 the expert system was revised by Dr M. Brinck, an expert system specialist, using the expert system shell dmX in a Windows environment. The revised expert system was called PEDSSys (Plant estuarine decision support system) to distinguish it from the first prototype.

The expert system shell used, dmX for Windows was developed locally by Decision Management Software. It is a product designed for handling, scoring and rating classification problems. dmX uses bilinear logic which was designed to model "fuzzy" attitudes, which are strictly speaking probabilities. Bilinear logic is recommended as the paradigm of choice for applications which require judgemental, attitudinal or probabilistic reasoning.

The inference engine of an expert system does the actual work by considering existing knowledge and adding information to the knowledge base until it reaches a conclusion. In dmX inference is based on an inference or concept net. Inference nets originated from

research into applying probabilistic reasoning in expert systems, the principles can be applied wherever "nodes" are not simply true or false, but take on some numeric value which is either directly entered by the user, or computed from the values of other nodes. In dmX, nodes can represent probabilistic statements, degree of belief, attitudes, numeric measurements and values which are numerically calculated. Since the values of the nodes or concepts may be calculated from the values of other concepts, concepts may be considered to be linked together in a network or concept net.

Program information

The program can be divided into Input, Inference or Scoring and Reports.

Input: The user is asked a series of questions which may require a qualitative or quantitative answer. Each input may have a different weight which may be positive or negative. For example if "water depth" comes in with a negative values, it could be multiplied by 0.2 (on a scale of 1 to -1 where 1 represents "very good" and -1 represents "very bad"). The questions asked by PEDSSys are shown in Table 12. The presence or absence of pollutants has been included in the expert system as a general question. However the effect of pollutants on estuarine plant communities requires in-depth research and knowledge refinement.

Scoring: Scoring is according to the inputs and weights that have been assigned to these parameters in the program.

User interface: The user interface is Microsoft Access. In the development of the program full use was made of the user friendly environment of Access so that even people with very limited computer experience would find the program simple to operate.

Expert system knowledge/rules

Two concerns for those using or considering the use of expert system tools is the development of the rule base and the logical structure for combining knowledge into a decision (Geselbracht and Johnston 1988). Constructing a meaningful knowledge base requires an investment of time into gathering and eliciting potential rules, combining those rules into a larger framework and calibrating the knowledge base to expert knowledge. A significant final step in the calibration of the knowledge base is to run the system with inputs for some typical problems that it will be expected to solve. The same problems should be given to an expert and their solutions compared. In this study, the knowledge was obtained from the literature and was expanded and refined from field work and controlled laboratory studies.

Table 12 Questions asked by the expert system PEDSSys.

SELECT PLANTS PRESENT:

Emergent macrophytes: *Phragmites australis*, *Scirpus maritimus*
Salt marsh macrophytes: *Juncus kraussii*, *Salicornia* spp., *Sarcocornia perennis*, *Spartina maritima*, *Sporobolus virginicus*, *Triglochin* spp.
Submerged macrophytes: *Potamogeton pectinatus*, *Ruppia cirrhosa*, *Zostera capensis*.
Phytoplankton: *Diatoms, dinoflagellates*
Benthic microalgae:

QUESTIONS FOR SUBMERGED MACROPHYTES

Choose which water level applies:
increased to 2.5 m or more
increased, but < 2.5 m
decreased without exposure
decreased with exposure, sediment remains saturated
decreased with exposure, sediment drying out

Turbidity: high/medium/low

Water velocity (m s^{-1}) ?

Salinity (ppt) ?
Salinity will be maintained for (days) ?

Sediment will remain saturated for (days) ?

Pollution is present: no/yes

QUESTIONS FOR SALT MARSH AND EMERGENT MACROPHYTES

Choose which water level applies:
risen to cover > 75% of plant
risen to cover 50-75% of plant
risen to cover < 25% of plant
dropped, but sediment remains saturated
dropped and sediment is drying out

Season: autumn/winter/spring/summer

Plants will remain covered for (days) ?

Salinity (ppt) ?
Salinity be maintained for (days) ?

Sediment will remain dry for how many days ?

Pollution is present: yes/no

QUESTIONS FOR PHYTOPLANKTON (DIATOMS AND DINOFLAGELLATES AND BENTHIC MICROALGAE

The sediment is: sand/silt/mud

Nitrate (µg/l) ?

Salinity: bottom (> 0.5 m water depth)
top (< 0.5 m water depth)
mouth
middle
head

The salinity will remain for (days) ?

Knowledge/rules for submerged macrophytes

All estuarine plants have an optimal/suboptimal salinity range of tolerance (Table 13). The optimal salinity range is that at which maximum plant production occurs. Within this range, the plants grow rapidly, flower and germinate. Within the suboptimal salinity range productivity is lower and plant cover sparse. The maximum time that plants can be exposed to lethal salinity levels is also considered. If the salinity is less than the suboptimum minimum or greater than the suboptimum maximum a negative score is obtained in PEDSSys according to time (0 to 30 days) on a linear curve. If salinity is between the optimums and suboptimums values, a negative score is obtained according to time (0 - 90 days) on a linear curve.

Submerged macrophytes will not persist in estuaries where the water current velocity is > 1 ms^{-1}. Current velocities > 1 ms^{-1} (which would represent flood conditions in South African estuaries) would remove submerged macrophyte beds. At 0.5 ms^{-1} growth would be significantly reduced and at water velocities < 0.1 ms^{-1} aquatic macrophytes would grow and establish themselves well.

Submerged macrophytes are sensitive to changes in water clarity determined by sediment load; their depth distribution is controlled by this factor. Turbidity is expressed as high, medium or low in the expert system. This parameter needs to be quantified more accurately as actual secchi disc or irradiance (μmol m^{-2} s^{-1}) readings. In the expert system, the depth limit for submerged macrophytes was set at 2.5 m. *Potamogeton pectinatus* L. has been found at 2.6 m in the Swartvlei (Howard-Williams and Allanson 1978) and 2.5 m in the Bot River estuary (Bally *et al.* 1985). Depth distribution is variable as it is dependent on turbidity and light penetration.

Zostera capensis can be exposed above water without drying for a longer time than can either *Ruppia cirrhosa* and *Potamogeton pectinatus* (Table 13). The resistance of the vegetative parts of *Ruppia* to drying is very low. After exposure, all plant parts except ripe seeds die within a few days (Verhoeven 1979). Monitoring studies in the Seekoei (33°05'S 24°55'E) and Kabeljous (34°00'S 24°56'E) estuaries have shown that, over a one month period, there was complete die-back of *Ruppia cirrhosa* beds following a drop in water level that exposed the beds. From this, the rule applied is shown in Table 13 (last two lines).

TABLE 13 Expert system knowledge for submerged macrophytes.

	Zostera capensis	*Ruppia cirrhosa*	*Potamogeton pectinatus*
Optimal salinity range (ppt):	10-40	< 30	< 15
Suboptimal salinity range (ppt):	0-10 40-45	30-60	15-30
Sub-optimal exposure time (months):	>1<3	< 1	< 1
Death exposure time (months):	>3	>1	>1

Knowledge/rules for emergent macrophytes

To date only two emergent macrophytes have been included in the expert system because data are not available for others. These are *Phragmites australis* (Cav.) Trin and *Scirpus maritimus* L. For *Phragmites australis* the optimal salinity range is < 15 ppt (Table 14). Benfield (1984) reported 100 % mortality of *Phragmites* after three months at 30 ppt. Our laboratory studies have shown that if *P. australis* roots and rhizomes are located in freshwater, the plants will survive tidal inundation with marine water. However plants with their roots in 20 ppt showed signs of stress after two weeks.

PEDSSys gives six different water level scenarios (Table 12) for emergent and salt marsh macrophytes. The season is also considered as plants will respond differently to various degrees of inundation at different times of the year. If plants are completely submerged they will not flower and produce seed. Flowering periods for the different macrophyte species are given in Table 15.

Emergent macrophytes require waterlogged conditions. If the water level is dropped and the sediment is drying out, as time increases the growth rate adjustment value given by PEDSSys becomes more negative. If the sediment is not waterlogged and there is no groundwater source, then the predicted response is the death of the plants after three months (Table 14).

TABLE 14 Expert system rules for emergent macrophytes.

	Phragmites australis	*Scirpus maritimus*
Optimal salinity range (ppt):	0-15	0-15
Suboptimal salinity range (ppt):	15-30	15-30
Time exposed to die salinity range before dying (days):	30	30
Soil not waterlogged death time (months):	3	3

Table 15 Flowering seasons for macrophytes included in the expert system.

SPECIES	FLOWERING SEASON
Juncus kraussii	late spring/summer
Phragmites australis	summer/autumn
Salicornia spp	summer/autumn
Sarcocornia perennis	summer/autumn
Scirpus maritimus	spring/summer
Spartina maritima	spring/summer/autumn
Sporobolus virginicus	spring/summer

Knowledge/rules for salt marsh macrophytes

Optimal and suboptimal salinity ranges for salt marsh macrophytes are given in Table 16. Salt marsh macrophytes predominate at a salinity between 10-30 ppt (Hoese 1967) or between 18-35 ppt (Odum 1988). *Spartina*, *Sarcocornia* and *Salicornia* appear to have an absolute requirement for salt (Clarke and Hannon 1970) as they die at < 5 ppt. However, these studies have shown that this is not true for *Sarcocornia perennis* (Miller) Scott as growth was stimulated under freshwater conditions. Optimum growths of *Triglochin* spp., *Sporobolus virginicus* (L.) Kunth. and *Juncus kraussii* Hochst. occur at lower salinity levels and they do not have an absolute requirement for salt (c.f. Table 16).

Other rules used with salt marsh macrophytes are their waterlogging and inundation tolerances. Although all salt marshes are subject to tidal immersion, tolerance to this varies between species. After three months submergence, salt marsh plants will die-back. Field monitoring in the Seekoei estuary showed that the *S. perennis* community died after three months complete submergence. Laboratory studies showed that after two weeks, growth of completely submerged plants was reduced by comparison with waterlogged treatments. Supratidal marsh plants such as *Juncus kraussii* may be more sensitive to immersion as it naturally occurs at higher elevations and is less frequently inundated. When data become available, this knowledge will need revision.

Salt marsh plants are adapted to withstand up to a full year of waterlogged conditions without any apparently adverse effects on growth. Clarke and Hannon (1970) found that *Triglochin* has an absolute requirement for waterlogged conditions. *Triglochin* plants will die after one month if the soil is not waterlogged (Table 16). Depending on salinity, the expert system predicts that salt marsh plants will only survive for six months if the sediment dries out.

Table 16 Expert system rules for salt marsh macrophytes.

	Spartina maritima	*Sarcocornia perennis*	*Salicornia* spp.
Optimal salinity range (ppt):	10-35	< 35	18-30
Suboptimal salinity range (ppt):	0-10 35-45	> 35	0-18 30-40
Time exposed to die salinity range before dying (days):	30	30	30
Sub-optimal submergence time (months):	>1<3	>1<3	>1<3

	Triglochin spp.	*Sporobolus virginicus*	*Juncus kraussii*
Optimal salinity range (ppt):	0-18	0-18	0-18
Suboptimal salinity range (ppt):	18-35	18-35	18-35
Time exposed to die salinity range before dying (days):	30	30	30
Sub-optimal submergence time (months):	>1<3	>1<3	>1<3

Knowledge/rules for phytoplankton and benthic microalgae.

Rules are used to establish whether, after the addition or abstraction of freshwater, phytoplankton blooms will occur in the estuary. The presence or absence of vertical and horizontal salinity gradients, the stability of the water column and the current speed are questions the expert system uses to establish this goal.

By asking for the mean bottom (< 0.5 m) and top (> 0.5 m) salinity the expert system establishes whether the estuary displays vertical salinity gradients. If the difference between the mean top and mean bottom salinity is greater than 5 ppt then a vertical salinity gradient exists. The rule is based on data comparing chlorophyll-*a* values and vertical salinity differences for several Cape estuaries.

By asking for salinity data for the mouth, middle and head reaches of the estuary, the expert system decides whether the estuary has a horizontal salinity gradient. If the mouth salinity > middle salinity > head salinity and the difference between the mean head and mean mouth salinity is greater than 10 ppt then a horizontal salinity gradient exists.

Although freshwater input may establish salinity gradients in the estuary a high freshwater nutrient input is also important to support phytoplankton blooms. Sampling in several Cape estuaries have shown that high concentrations of nitrate at the head of the estuary are correlated with high phytoplankton biomass. In the Sundays estuary consistently high nutrient loading occurs in the freshwater. High chlorophyll-*a* concentrations (defined as greater than 20 $\mu g\ l^{-1}$) can only be maintained in this estuary if the nitrate concentration is greater than 200 $\mu g\ l^{-1}$. The retention time of the water within the estuary is also important. Hilmer and Bate (1991) found that phytoplankton blooms formed in the upper reaches of the Sundays estuary when there was a water residence time of 6-7 tidal cycles (3-4 days, Mackay and Schumann 1990). The stable tidal conditions allowed the phytoplankton (especially dinoflagellates) to bloom as the conditions were present for longer than the doubling time requirements of most species. A similar situation, where freshwater input results in consistent high nutrient levels and, therefore, large phytoplankton biomass occurs in the Great Fish estuary (33°29'S 27°06'E) Mean chlorophyll-*a* in the Great Fish estuary has been reported as 20.5 $\pm$ 31.5 $\mu g\ l^{-1}$ (Allanson and Read 1987).

Knowledge of the salinity stratification of the estuary is important as it can be used to predict species composition. Dinoflagellates are abundant in stratified waters whereas diatoms are abundant in well-mixed waters.

The preliminary work on benthic microalgae of Cape estuaries indicate that even where conditions are not suitable for phytoplankton, a high biomass of benthic chlorophyll-*a* may be present (Rodriguez 1993). Benthic microalgae biomass is highest in mud sediments

compared to sand and silt sediments. Table 17 shows the growth rate adjustment values (between 0 and 10) given by PEDSSys for benthic microalgae under different nutrient and sediment conditions.

Table 17 PEDSSys growth rate adjustment values for benthic microalgae under different nutrient and sediment conditions.

	SEDIMENT TYPE		
WATER COLUMN NITRATE (µg/l)	SAND	SILT	MUD
25	0	1.3	5.0
50	0	3.8	7.5
100	0.5	4.2	8.0
200	1.5	5.2	9.0
300	2.5	6.3	10.0

Model validation

The expert system is validated by applying the system to a specific estuary. Within the Co-ordinated Estuarine Research and Management (CERM) group, co-ordination of existing estuarine models was conducted in 1993 through their application to a case study, namely the Great Brak estuary (Fig. 11).

Run-off scenarios were chosen for model application i.e. the natural run-off situation versus the full demand situation. EMATEK (Division of Earth, Marine and Atmospheric Science and Technology, Council for Scientific and Industrial Research) used the hydrodynamic model Mike-11 to predict the new salinity and water levels in the estuary. With this data EDSSys was used to predict the effect on the plant communities. After modifications to the Mike-11 data such as monthly averaging of salinities, EDSSys produced useful qualitative predictions of the estuarine floral response (Slinger 1994).

For the future management of estuaries, we propose that a hydrodynamic model is the first requirement since it will determine the physical characteristics of the system; the output from this should then be utilised by expert systems aimed at predicting the responses of estuarine flora and fauna and social consequences.

Future development of estuarine plant models

Future extensions to the expert system could include the addition of more plants to existing groups (i.e., salt marsh, emergent macrophytes) as information becomes available. Plants could be added as new groups, i.e. mangroves and macroalgae. Other considerations are the addition of a graphic prompt. It may be possible to scan in diagrams or photographs of plants that would assist visual recognition by non-botanists.

The expert system includes the ecophysiological tolerances of estuarine plants and the response of phytoplankton to freshwater input. It does not predict changes in plant community structure due to competition, succession or interaction with organisms. Dynamic factors such as the rate of establishment and recovery of plants after perturbation are also excluded. A future extension to the modelling of estuarine plants would involve geographical information systems where the plant's distribution and habitat can be mapped.

A limitation with the expert system approach is that consequences of reduced freshwater input are predicted, but the rates at which they occur are not predicted. In collaboration with Prof J. Hearne (Department of Maths and Applied Maths, University of Natal, Pietermaritzburg) a dynamic vegetation model is being developed under the auspices of CERM. In the period between May 1993 and March 1994, a non-spatial, biomass growth model for estuarine macrophytes was developed and tested on a preliminary range of scenarios. The preliminary results showed the potential of such a modelling tool to predict the time-dependent response of estuarine vegetation over the long term and the need to extend this capability to incorporate spatial change. A spatial model based on GIS (Geographical Information Systems) principles will be developed. Plant community changes in response to physical conditions could then be mapped and geographically displayed. An example of this is the spread of emergent macrophytes in response to increased sedimentation and decreased salinity.

Conclusions

As information becomes available the expert system knowledge can be revised. The present knowledge represents the predicted effect on the plant based on the best available data. The present generation expert systems are far from "expert" in their level of performance, as they support only simple knowledge structures and primitive inferencing capabilities (Davis *et al.* 1989). However, from a manager's point of view, these systems, at least, provide advantages over traditional computer programs. The main reason for this is that expert systems can assess qualitative information and give valid logical conclusions. In this way the estuarine plant expert system can be useful to water managers because it combines information on the responses of estuarine plants to freshwater. It can be used as a predictive tool for different freshwater input scenarios.

8. GENERAL CONCLUSIONS AND RECOMMENDATIONS

The expert system, PEDSSys (Plant Estuarine Decision Support System) was developed to provide a predictive capability for estuarine plant response to varying freshwater inputs. PEDSSys combines information on estuarine plant response to the physical factors related to freshwater, potentially making it a useful component in water management decisions.

Plants included in the expert system were divided into four groups based on habitat types viz.: phytoplankton, submerged, salt marsh and emergent macrophytes. Each habitat type has a different freshwater environment and, therefore, different rules were used to describe the plants' responses to freshwater. Field studies and controlled laboratory experiments were conducted to obtain information for the expert system rules.

Freshwater inflow controls many physical factors to which the plants respond, namely: salinity, flow velocity, water level, sedimentation, increased frequency of mouth closure and reduced nutrient input. The importance of freshwater to the survival of estuarine plants is discussed in relation to these physical factors.

A primary consequence of reducing freshwater input is increased water column salinity due to evaporation and a reduction in the dilution of seawater by freshwater. Freshwater management decisions have to consider the maximum time that plants can be exposed to elevated salinities before they die. This is, therefore, included as an expert system rule. Controlled laboratory experiments have shown that the seagrass *Zostera capensis* dies after three months at a salinity of 55 ppt and after one month at a salinity of 75 ppt.

Maximum growth of *Zostera capensis* was at a salinity of 15 and 35 ppt. Growth of *Ruppia cirrhosa* continued at salinity ranges between 0-75 ppt, but with maximum growth in freshwater. Despite its wide range of salinity tolerance, *R. cirrhosa* was found in brackish estuaries where the salinity was less than 30 ppt. *Zostera capensis* was common in estuaries with open mouths, characterized by marine conditions (35 ppt). Because of its stronger morphological structure, *Z. capensis* has a competitive advantage over *R. cirrhosa* in estuaries where there is tidal exchange and stronger water currents. When plants of the two species were grown in the same tank, *R. cirrhosa* expansion and growth was greater than *Z. capensis* at both 15 and 35 ppt. This was true for the experimental conditions of reduced water velocity and constant temperature and irradiance.

In estuarine environments, the ability of plants to tolerate fluctuating salinity is important. *Ruppia cirrhosa* is an opportunistic species that can tolerate fluctuating salinity. It was found to recover after exposure to high salinity. This is associated with the accumulation of the osmotic solute proline.

Ruppia cirrhosa is adapted to its habitat in periodically open estuaries because of its

ability to tolerate fluctuating salinity and recover from high salinity. Erratic freshwater input into estuaries causes salinity to fluctuate between brackish and hypersaline conditions. Seed germination, however, has a different salinity optimum to that of vegetative growth. No seeds germinated where the salinity was greater than 55 ppt. In the presence of seeds, a freshwater pulse into an estuary would likely reduce hypersalinity and promote *R. cirrhosa* germination and growth. Hence the negative effects of hypersalinity can be corrected by allowing the system to be flushed with water of low salinity.

Lack of freshwater inflow into the Kromme estuary has resulted in high water column salinity that has caused salt accumulation in the intertidal marshes (Adams *et al*. 1992). Marsh productivity is known to decrease as salinity increases. This study has shown that where the salinity is greater than 35 ppt, reduced growth of *Sarcocornia perennis* and *Spartina maritima* is the consequence. Freshwater input, is therefore, important for the survival of these plants as it reduces high water column salinity. Freshwater also dilutes sediment salinity, preventing dry hypersaline conditions that reduce macrophyte germination and growth (Price *et al*. 1988).

When rivers are impounded there is a reduction in the longitudinal salinity gradient between the mouth and the head of the estuary. This has occurred in several eastern Cape estuaries that have merely become "side arms of the sea" with seawater extending into the upper estuarine reaches. Lack of freshwater has reduced macrophyte diversity and there is evidence that marine submerged macrophyte communities have extended into the upper reaches of these estuaries and displaced the brackish communities (Adams and Talbot 1992, Adams *et al*. 1992).

To maintain estuarine plant communities the salinity gradient in an estuary must be preserved. A permanent increase in salinity to above 25 ppt will result in a loss of the brackish plant community. If the salinity is allowed to increase to above 45 ppt the salt marsh habitats will be adversely affected.

Localized freshwater inputs from small tributaries leading into estuaries are also important as they provide sites for nodes of plant and animal diversity. Small tributaries often are too small to influence the salinity of the surface water, but they do influence the salinity of interstitial water. Plants at these sites can range from salt tolerant to brackish species. Brackish reeds such as *Phragmites australis* are excellent indicators of freshwater seepage points in estuaries. Laboratory studies have shown that *P. australis* can survive tidal inundation with seawater (35 ppt) if the root system is located in low salinity or freshwater. Freshwater impoundment in small farm dams and the utilization of aquifer water in the lower tributaries of estuaries may be causing increased salinization of surface water and decreased freshwater run-off into estuaries. This could adversely affect the survival of macrophytes in these systems. On the other hand, these small farm dams

may be extending the seepage duration of tributaries. This would extend the period of fresh interstitial water and maintain species diversity in small nodes under conditions that would otherwise become saline. This aspect is worthy of further hydrological modelling.

A decrease in freshwater flow can result in increased submerged macrophyte growth. One general rule used in the expert system is that submerged macrophytes will not persist in estuaries where the water current speed is > 1 ms^{-1}. Adequate South African literature (Branch and Day 1984, Howard-Williams and Allanson 1978) and overseas data are available to support this.

Lack of freshwater flow also reduces flushing and dilution of wastes. A decline in the water quality of an estuary can result in blooms of algae such as *Enteromorpha* and *Cladophora*, (Johnston 1971, Josselyn and West 1985) which is aesthetically unacceptable. Macroalgal blooms have already been shown to adversely impact the social acceptability of water in the Great Brak estuary.

In periodically closed estuaries during times of mouth closure, a reduction in freshwater input results in a drop in water level. Submerged macrophytes are exposed and die-back. Studies monitoring the periodically closed Seekoei and Kabeljous estuaries have shown that, over a one month period, there was complete die-back of *Ruppia cirrhosa* beds following a drop in water level that exposed the beds. Laboratory studies showed that *R. cirrhosa* was more sensitive to desiccation than was *Z. capensis*. *Ruppia cirrhosa* plants exposed for 2h took four days to recover, whereas *Z. capensis* plants recovered within one day.

In South African estuaries *R. cirrhosa* is notably absent from intertidal habitats. Our experimental studies showed that *Z. capensis* can withstand repeated cycles of drying during daily low tides and can, therefore, possibly outcompete *R. cirrhosa* in intertidal habitats. There was no significant difference between the leaf production of *Z. capensis* for submerged plants and those exposed for 5h daily. However, a daily 5h exposure was lethal for *R. cirrhosa*. The resistance of the vegetative parts of *Ruppia* to drying is very low. After exposure, all plant parts except ripe seeds, die within a few days (Verhoeven 1979).

A drop in water level will also affect the emergent and salt marsh macrophytes as they require waterlogged conditions. Abundant soil water plays an important role in maintaining high productivity. The water is used directly by the plants but also holds the nutrients in a dissolved state in the sediment (Keefe 1972).

Laboratory studies showed that the growth of *Sarcocornia perennis* was reduced in damp

treatments compared to saturated treatments. Dry treatments inhibited growth of *Spartina maritima* showing that plant's requirement for waterlogged conditions. As a result, the expert system rule states that if the sediment is not waterlogged and there is no groundwater source, then emergent reeds and sedges will die after one month and salt marsh plants after six months. However, plant response is also dependent upon the prevailing salinity conditions that can be influenced by rainfall events. The amount of rain may not be sufficient to alter estuary water salinity, but may be adequate to wash salt out of surface soil layers.

Freshwater, as a flood affects sediment transport within an estuary and condition of the mouth. Seawater imports sand into the mouth during each tidal cycle and this is only scoured out by floods. The construction of dams and the abstraction of freshwater prevents this periodic scouring action. The result of dam construction, therefore, is sediment accumulation in the subtidal zone and deposition of flood tidal deltas (Reddering 1988).

Submerged macrophytes are adapted to a variable freshwater input. *Zostera capensis* beds may disappear completely after a major flood, but generally re-establish after a period of 1-3 years (Talbot *et al*. 1990). Dams reduce the effects of floods. A consequence of this is an increase in the growth and expansion of submerged macrophytes because of increased sediment stability and the improved water clarity that is related to reduced freshwater input. Studies in the Kromme estuary have shown that the areal cover and biomass of *Zostera capensis* has increased since the construction of a second dam (Charlie Malan) in the catchment (Adams and Talbot 1992).

Adequate freshwater input ensures that the mouth of the estuary remains open, allowing tidal exchange. Tidal exchange is important as salt marsh plants are alternately drained and inundated by tidal action. Salt marsh plants occur in distinct zones along an elevation and tidal inundation gradient as one moves away from the water. *Spartina maritima* generally occurs at the low tide level succeeded by *Sarcocornia perennis*, *Triglochin* spp., *Limonium* spp., *Chenolea diffusa*, *Sporobolus virginicus* and *Sarcocornia pillansii* at higher, drier levels. If the tidal range is restricted, for example by mouth closure, then the zonation and diversity of plants is lost. Tidal exchange is also important as the flushing of salt marshes prevents dry hypersaline conditions that reduce plant productivity and germination.

Although salt marsh plants require tidal flushing, they are sensitive to inundation and continual flooded conditions. In European salt marshes which experience regular flooding there are rarely more than 10 species present, whereas for areas that are only flooded monthly, up to 40 species occur (Odum 1988). In periodically open estuaries in South

Africa eg. the Great Brak and Kabeljous (Fig. 1) during times of mouth closure, water levels rise and salt marshes may be inundated for extended periods which results in a die-back of plants.

Laboratory studies on *Sarcocornia perennis*, have shown that the plants are stressed two weeks after inundation. Growth of completely submerged plants was reduced by comparison with waterlogged treatments. Completely submerged plants decomposed rapidly in low salinity treatments.

If management options are considered, prolonged inundation of *S. perennis* marshes should be avoided during the growing season (spring/summer). *Sarcocornia* will not flower nor will it produce seed when inundated (O'Callaghan 1992). This is significant as it would reduce the potential for recruitment of new plants when water levels eventually subside.

In high rainfall regions, if the tidal flushing of salt marshes is restricted for extended periods, sediment salinity will be reduced allowing encroachment by brackish reeds or terrestrial species into previous salt marsh areas. Typical salt marsh mosaics may be converted to essentially pure stands of *Phragmites* thus reducing the biotic diversity of the system. In many of our east coast estuaries the intertidal zone has been lost due to sedimentation caused by soil erosion in the catchment and due to tidal restriction. This has led to *Phragmites australis* encroachment and a loss of habitat diversity. There is a need to conserve southern African salt marshes as O'Callaghan (1990) has shown how poorly developed the halophytic vegetation is around the False Bay coast due to human impacts and reduced saline inputs. Conservation of salt marshes is therefore dependent on maintaining adequate tidal exchange and freshwater input.

The amount of freshwater input determines the dominance of either phytoplankton or macrophytes as the major primary producers. Phytoplankton dominance in estuaries has been associated with increases in freshwater inflow both in southern Africa and overseas. In southern Africa phytoplankton are dominant in large channel-like estuaries (eg, Sundays and Gamtoos) which have large catchments and therefore a large mean annual run-off and freshwater input. The nutrients derived from freshwater support the high phytoplankton biomass. Reduced freshwater inflow into an estuary favours submerged macrophytes as there is a decrease in turbidity and water velocities and a more stable sediment and salinity environment.

The estuary is dependent upon salt marshes, riparian vegetation and river flow to sustain its particulate food resources (Allanson 1992). Any changes in the plant habitat will lead to changes in the secondary productivity of the estuary and the nature and status of the system.

9. RECOMMENDATIONS FOR FUTURE RESEARCH

1. Testing and further development of PEDSSys is required.

It is important that the new expert system, PEDSSys is tested and validated. We suggest that the knowledge in the expert system is tested by other estuarine botanists or ecologists, namely Dr Ricky Taylor, Natal Parks Board; Dr Brian Allanson, Allanson and Associates; Prof Steinke and Prof Naidoo, University of Durban-Westville. After testing the expert system needs to be revised and made available to water managers. If PEDSSys is found to be useful, then additional salt marsh plants or new groups of plants, i.e. macroalgae and mangroves should be added to it. The addition of a graphical prompt, including plant diagrams would make the identification of plants by the expert system user much easier.

2. Studies expanded to include Natal estuaries and species.

The macrophyte section of this study focused on estuaries and plants specific to the Cape Province. Studies should be extended to include the estuaries in Natal and the importance of freshwater for subtropical species such as mangroves.

Future research should concentrate on the ecophysiological responses of estuarine macrophytes not yet included in the plant expert system. Adult plants were used in this study; however, it is also important to determine the effect of salinity and inundation on seedling establishment and growth. Knowledge of the ecophysiological tolerances of plants at all phases of the life-cycle is required. Field monitoring is needed to validate laboratory studies.

3. Development of spatial vegetation models.

Knowledge of the functioning and freshwater requirements of estuaries has improved but still lacks integration. A future research requirement is the inclusion of a dynamic link between vegetation and the environmental factors that cause change, so that processes of growth and colonisation of vegetation can be modelled. The future of plant modelling is Geographical Information Systems (GIS) which will allow dynamic processes to be mapped. Information on estuarine macrophyte dynamics such as rates of establishment and recovery after perturbations is required. GIS modelling can include freshwater, salinity and topographical relationships.

4. Identification of phytoplankton and benthic microalgae species and development of an identification key for South African estuaries.

In order to understand estuarine ecology, the scientific names of the species have to be known. We have begun to develop an identification key for South African estuarine species. However this is a long and tedious process and funds are needed to continue this work. Microalgal identification must be used to establish whether there are characteristic estuarine species not normally found in the open sea or the freshwater of rivers, and the part they play in estuarine ecology.

5. Research on benthic microalgae.

The preliminary work on benthic microalgae in this study has shown that it is a very important in south-Cape estuaries. Work should continue to establish the importance of benthic microalgae in South African estuaries, their response to freshwater inflow and their contribution to total primary production. It is suggested that primary production is estimated using oxygen microelectrodes. The clear advantage of the microelectrode technique over traditional ^{14}C and O_2 exchange, is that the sediment column can be analyzed every millimetre. This will help to understand primary productivity processes within the photic and the non-photic zone.

6. Salt marsh studies

In order to integrate physical models (eg. estuarine hydrodynamic models) and the estuarine plant expert system, research on estuarine plant responses to freshwater controlled physical factors must continue. An essential component of South African estuaries is the salt marsh. An understanding of the interaction between the salt marsh and the physical environment are essential if management of the freshwater requirements of an estuary is a goal. Destruction of the salt marsh will lead to a deterioration in faunal habitat. Studies on the effect of water level changes on salt marsh productivity should be extended.

Reduced freshwater input results in sedimentation and the formation of floodtidal deltas. Research should focus on the relationship between reduced freshwater input, sedimentation and loss of salt marsh areas. The question whether *Spartina maritima* is an exotic that is spreading and increasing the rate of sedimentation in our estuaries (Allanson 1992) needs to be answered.

Future research should aim at determining the role of rainfall and tidal inundation in

regulating salt marsh salinites because a lack of freshwater flooding and the resulting hypersaline conditions in salt marshes may cause a threat to the estuarine environments as we know them today.

7. Freshwater seepage

Estuaries are dependent on freshwater input from the larger catchment. however rainfall and run-off below impoundments can also influence estuarine salinities. The importance of freshwater seepage from tributaries leading into estuaries needs to be quantified. This is important as freshwater input from these localized sources may prevent hypersaline conditions and impoverishment of an estuary during times of drought. Small farm dams may be extending seepage duration of tributaries. This is important as the period of fresh interstitial water is extended under conditions that would otherwise become saline. Nodes of species diversity are created i.e. plants present range from brackish to salt tolerant species.

8. Co-ordination of hydrodynamic studies and phytoplankton responses.

To provide a better understanding of phytoplankton response to freshwater inflow, a co-ordination of hydrodynamic studies and phytoplankton response is required. According to Cloern (1991a) realistic simulation of phytoplankton populations is dependent upon accurate descriptions of mixing processes in estuaries.

This study has shown that short term increases in the quantity of freshwater input, causes and increase in nutrient levels and phytoplankton. These freshwater pulses may be important in maintaining the pelagic food chain in certain estuaries. This needs to be investigated.

9. Linking floral and faunal responses to freshwater input.

Research on estuarine flora must be integrated with the faunal component so that a community response to freshwater controlled physical factors can be identified. This will contribute to an understanding of the ecological dynamics of estuaries. This study has attempted to establish IF-THEN scenarios but the question SO-WHAT? nas to be answered before effective management of estuaries can take place.

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APPENDIX

MICROALGAL IDENTIFICATION : SPECIES LISTS

Table 1 Phytoplankton (diatom) species found in Cape estuaries sampled. (B = Berg, Ga = Gamtoos, G = Gouritz, GB = Great Brak, GF = Great Fish, KF = Kafferkuils, KB = Keurbooms, P = Palmiet and S = Sundays).

SPECIES	ESTUARIES								
	B	Ga	G	GB	GF	KF	KB	P	S
Actinoptychus splendens			•			•	•		
Actinoptychus undulatus						•			
Amphiprora sp.	•					•	•		
Amphora coffeaeformis				•		•	•		
Amphora sp.	•	•	•	•		•	•	•	•
Anaulus sp.1			•	•					
Anaulus sp.2			•			•			
Asterompheus sp.							•		
Asterionella glacialis							•		
Auliscoseira sp.	•		•			•		•	
Auricula sp.			•						
Bacillaria sp.	•					•			
Biddulphia alternans	•	•					•		
Biddulphia tuomeyi						•	•		
Biremis sp.			•						
Campylodiscus sp.	•			•			•		
Caloneis sp.			•	•		•			
Chaetoceros curvisetus							•		
Chaetoceros protuberans							•		
Chaetoceros sp.1			•	•		•	•		
Chaetoceros sp.2							•		
Chaetoceros tripes							•		
Cymatosphenia sp.						•			
Cocconeis sp.1	•	•	•	•		•	•	•	
Cocconeis sp.2				•		•	•		
Cocconeis sp.3	•	•	•	•			•	•	
Cocconeis sp.4			•	•			•	•	
Cocconeis sp.5		•	•	•		•		•	
Cocconeis sp.6	•			•		•	•	•	
Coscinodiscus nodulifer		•	•			•	•		
Coscinodiscus radiatus	•	•				•	•		
Coscinodiscus sp.1			•	•		•			
Coscinodiscus sp.2			•	•					
Coscinodiscus sp.3		•		•		•	•		
Cyclotella sp.	•	•			•				
Delphineis sp.						•			
Diploneis didyma		•							
Diploneis sp.	•		•				•		
Ditylum brightwellii		•				•	•		
Entomoneis alata			•						
Entomoneis sp.	•		•	•		•			
Eucocconeis sp.						•			
Extubocellulus spinifera	•	•	•	•	•		•	•	•
Fragilaria sp.						•	•		
Grammatophora sp.							•		
Gyrosigma fasciola		•		•			•	•	
Haemaulus sp.				•		•	•		
Lauderia sp.		•		•			•		
Leptocylindrus sp.1	•	•		•		•	•	•	
Leptocylindrus sp.2	•			•		•	•		

Table 1 continued.

SPECIES	ESTUARIES								
	B	Ga	G	GB	GF	KF	KB	P	S
Licmophora sp.						*	*		
Lyrella sp.								*	
Melosira moniliformis	*								
Melosira nummuloides	*			*		*	*		
Melosira sp.1				*					
Melosira sp.2		*		*		*	*		
Navicula sp.1	*	*	*	*		*	*	*	
Navicula sp.2	*								
Navicula sp.4	*								
Navicula sp.5	*	*							
Navicula sp.6	*	*	*	*		*	*	*	
Navicula sp.7	*	*	*	*		*	*	*	
Navicula sp.8	*	*	*	*	*	*	*	*	
Navicula sp.9	*	*	*	*		*	*	*	*
Navicula sp.10	*	*	*	*	*	*	*	*	
Navicula sp.11	*	*		*		*	*		
Navicula sp.12	*								
Navicula sp.13	*								
Navicula sp.14	*		*	*				*	
Navicula sp.15	*								
Navicula sp.16	*								
Navicula sp.17			*						
Navicula tripunctata	*	*	*	*		*	*		
Nitzschia closterium	*	*	*	*		*	*	*	
Nitzschia constricta	*			*	*				
Nitzschia delicatissima	*		*	*		*	*		
Nitzschia longissima	*	*	*	*			*		
Nitzschia pacifica				*					
Nitzschia serriata	*			*		*	*		
Nitzschia sp.1	*	*	*	*		*	*	*	
Nitzschia sp.2			*						
Nitzschia sp.3			*						
Opephora sp.								*	
Petrodictyon sp.			*			*			
Petroneis sp.			*						
Psammodictyon sp.						*			
Pteroncina sp.						*			
Rhizosolenia sp.		*	*	*			*		
Rhizosolenia stolf						*	*		
Semiinavis sp.				*					
Skeletonema sp.							*		
Surirella sp.	*		*		*	*			
Synedra sp.				*			*		
Tabularia sp.	*	*	*	*	*	*			
Thalassionema nitzschioides		*	*			*	*		*
Thalassiosira decipiens	*			*		*	*	*	
Thalassiosira rotula						*	*		
Thalassiosira sp.		*	*	*		*	*		
Thalassiosira subtilis							*		
Thalassiothrix sp.				*			*		

Table 2 Benthic microalgae (diatom) species found in Cape estuaries sampled. (B = Berg, Ga = Gamtoos, G = Gouritz, GB = Great Brak, GF = Great Fish, KF = Kafferkuils, KB = Keurbooms, P = Palmiet and S = Sundays).

SPECIES	ESTUARIES								
	B	Ga	G	GB	GF	KF	KB	P	S
Amphiprora sp.		*				*	*		
Amphora coffeaeformis						*		*	
Amphora sp.		*	*	*	*	*	*	*	*
Auliscoseira sp.	*					*	*		
Bacillaria sp.	*								
Biddulphia alternans		*				*	*		
Campylodiscus sp.							*		
Cocconeis sp.1	*						*		*
Cocconeis sp.3						*			
Cocconeis sp.4							*	*	
Cocconeis sp.5							*	*	
Cocconeis sp.6							*		
Coscinodiscus nodulifer						*	*		
Coscinodiscus sp.3							*	*	
Cyclotella sp.	*		*						
Cymbella sp.				*					
Delphineis sp.							*		
Diploneis didyma			*						
Diploneis sp.							*	*	*
Entomoneis sp.						*		*	*
Entomoneis tubocellulus spinifera	*	*	*						
Fragilaria sp.							*	*	
Grammatophora sp.							*		
Leptocylindrus sp.1				*			*		
Licmophora sp.	*								
Melosira moniliformis							*		
Melosira sp.1							*		
Melosira sp.2	*	*		*		*	*	*	*
Navicula sp.1	*	*	*	*	*	*	*	*	*
Navicula sp.2							*		
Navicula sp.3								*	
Navicula sp.6	*								
Navicula sp.7	*	*							*
Navicula sp.8	*			*					
Navicula sp.9	*							*	
Navicula sp.10	*							*	
Navicula sp.13						*	*		
Navicula sp.17								*	
Navicula tripunctata	*								
Nitzschia closterium	*			*		*		*	*
Nitzschia constricta	*	*	*	*	*	*		*	*
Nitzschia delicatissima				*					
Nitzschia longissima				*					
Nitzschia seriata							*		
Nitzschia sp.1		*	*	*		*	*	*	*
Nitzschia sp.2						*			
Parlibellus sp.							*		
Seminavis sp.				*					
Tabularia sp.				*		*		*	
Thalassiothrix sp.							*		

Table 3 Species associated with marine (20-35 ppt.), brackish (5-20 ppt.) and freshwater (< 5 ppt.).

SPECIES	MARINE	BRACKISH	FRESH
Actinoptychus splendens	*		
Actinoptychus undulatus	*		
Amphitetras sp.	*	*	
Amphora coffeaeformis	*	*	*
Amphora sp.	*	*	*
Anaulus sp.1	*		
Anaulus sp.2	*		
Asterompheius sp.	*		
Asterionella glacialis	*		
Auacoseira sp.	*	*	*
Auricula sp.	*		
Bacillaria sp.	*		
Biddulphia alternans		*	
Biddulphia tuomeyi		*	
Bivirma sp.			*
Campylodiscus sp.	*	*	*
Catacombas sp.	*		
Chaetoceros curvisetus	*		
Chaetoceros protuberans	*		
Chaetoceros sp.1	*	*	
Chaetoceros sp.2	*		
Chaetoceros tripes	*		
Climacodyfena sp.	*		
Cocconeis sp.1	*	*	*
Cocconeis sp.2	*	*	*
Cocconeis sp.3	*	*	
Cocconeis sp.4	*		
Cocconeis sp.5	*	*	*
Cocconeis sp.6	*	*	*
Coscinodiscus nodulifer	*	*	
Coscinodiscus radiatus		*	
Coscinodiscus sp.1		*	
Coscinodiscus sp.2		*	
Coscinodiscus sp.3	*	*	*
Cyclotella sp.	*	*	
Cymbella sp.	*	*	
Delphineis sp.	*	*	
Diploneis didyma		*	
Diploneis sp.		*	*
Ditylum brightwelli	*		
Eunotia praerupta			*
Eunotia sp.	*	*	*
Fallacieuus spinifera	*	*	*
Fragilaria sp.		*	
Gunardia fascia	*		
Haemaulus sp.	*	*	*
Lauderia sp.	*		
Leptocylindrus sp.1	*	*	*
Leptocylindrus sp.2	*	*	
Lyrella sp.	*		
Melosira moniliformis		*	
Melosira nummuloides		*	
Melosira sp.1			*
Melosira sp.2	*	*	*
Navicula sp.1	*	*	
Navicula sp.2	*		

Table 3 continued.

SPECIES	MARINE	BRACKISH	FRESH
Navicula sp.3	*	*	
Navicula sp.4		*	*
Navicula sp.5			*
Navicula sp.6	*	*	*
Navicula sp.7	*	*	
Navicula sp.8	*	*	
Navicula sp.9	*	*	*
Navicula sp.10	*	*	*
Navicula sp.11	*	*	
Navicula sp.12		*	
Navicula sp.13		*	
Navicula sp.14	*	*	
Navicula sp.15		*	*
Navicula sp.16			*
Navicula sp.17		*	
Navicula bipunctata	*	*	*
Nitzschia closterium	*	*	*
Nitzschia constricta	*	*	
Nitzschia delicatissima	*	*	
Nitzschia longissima	*	*	
Nitzschia pacifica	*		
Nitzschia seriata	*		
Nitzschia sp.1	*	*	*
Nitzschia sp.2			*
Nitzschia sp.3			*
Petrodictyon sp.	*		
Petroneis sp.	*		
Psammodictyon sp.		*	
Pteroncola sp.	*		
Rhizosolenia sp.	*		
Rhizosolenia stolf	*	*	
Seminavis sp.	*		
Skeletonema sp.	*		
Surirella sp.			*
Tabularia sp.	*	*	*
Thalassionema nitzschioides	*	*	
Thalassiosira decipiens	*	*	*
Thalassiosira rotula		*	*
Thalassiosira sp.	*	*	
Thalassiosira subtilis		*	
Thalassiothrix sp.	*