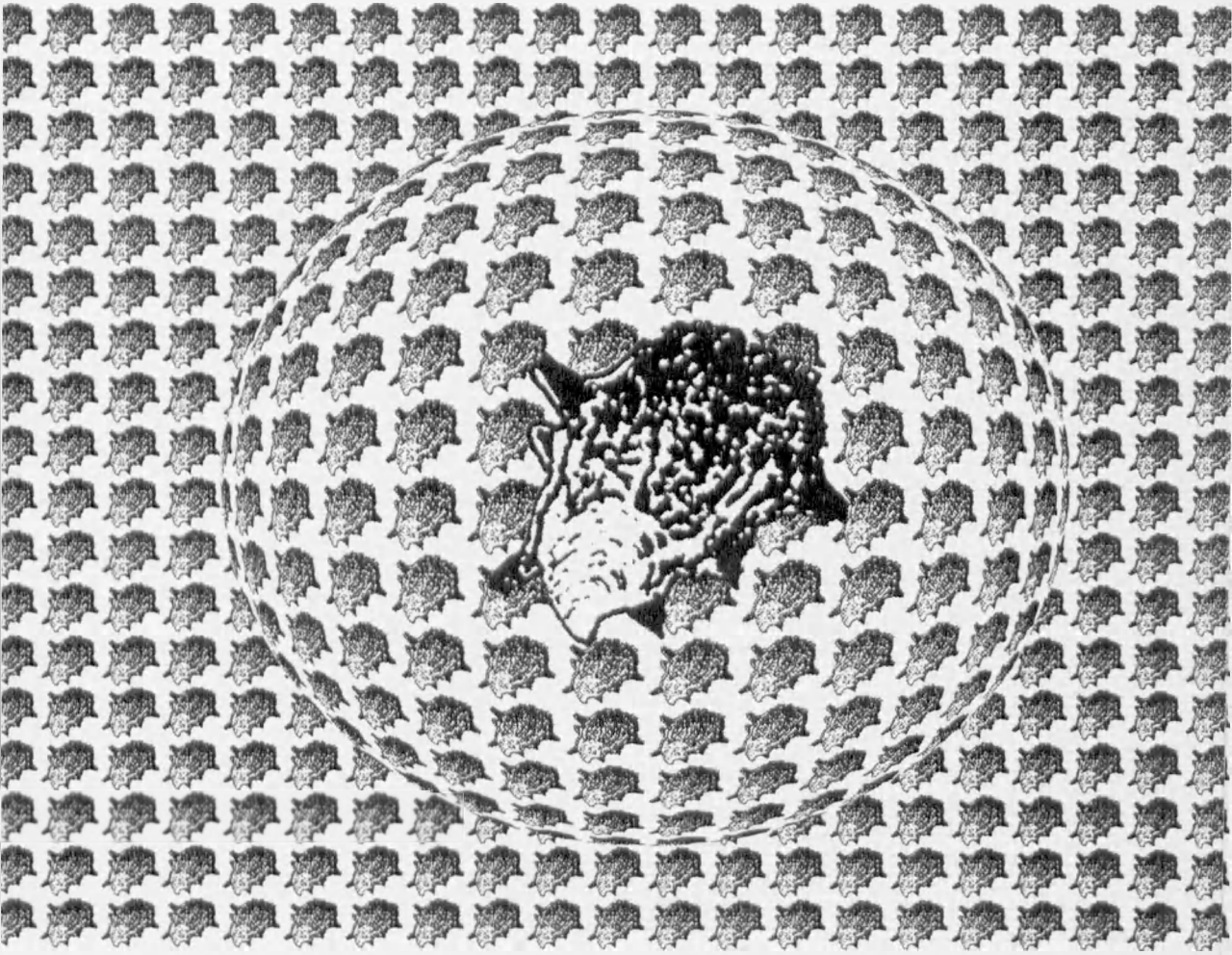
Behavioural aspects of the mangrove oyster *Saccostrea cucullata* (von Born, 1778) explaining its macro and micro distribution along the Kenyan coast



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Vrije Universiteit Brussel

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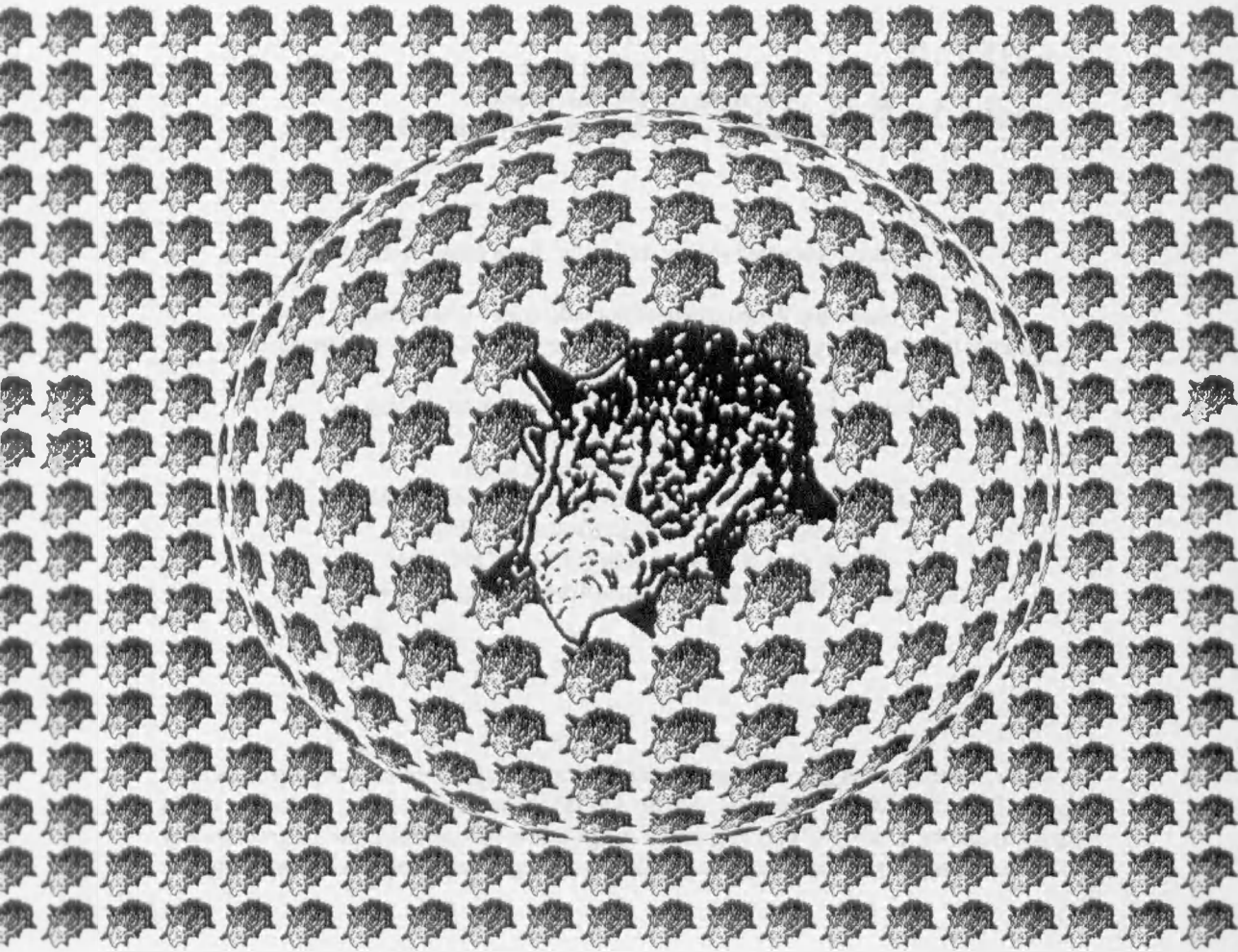

J.F. Tack

Vrije Universiteit Brussel

Studie voorgelegd aan de Koninklijke Academie voor Overzeese Wetenschappen
voor de wedstrijd 1999:
'Een studie over de ecologie van de Afrikaanse mangroven of over de biologie van de bestanddelen van hun flora of hun fauna'

J.F. Tack
Vrije Universiteit Brussel
Faculteit der Wetenschappen
Laboratorium voor Ecologie en Systematiek

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This studys was written as a collection of 9 articles. Those articles were written in function of submission to scientific journals. Because of the different guidelines to authors (titles and subheadings, artwork, figure legends, references, abbreviations, acknowledgements, etc. ...), used by different publishers/editors, the articles in this study show minor differences in their layout.

To Mzee
For a life full of ideas

CONTENTS

Preface

This study tries to explain how a number of mechanisms, acting on different levels, influences the distribution of *Saccostrea cucullata* along the Kenyan coast. Figure 1 shows an overview of the different relations studied.

The availability of fresh water, or at least the temporarily availability of fresh water, plays a central role in the explanation of the macro distribution of the mangrove oyster *S. cucullata.*
The presence of fresh water is closely related with the distribution of the mangrove ecosystem along the Kenyan coast. It is generally accepted that the mangrove distribution is closely linked with the presence of a brackish water micro environment caused by river discharges into the oceans. This study will show the importance of groundwater as an additional or alternative source of fresh water causing the brackish water micro environment needed for the development of the mangrove ecosystem along the Kenyan coast.
We will show that the relative low abundance of plankton in tropical coastal ecosystems forces *S. cucullata* to make use of mangrove detritus as an additional food source.
The combined effect of the availability of fresh water in the mangrove ecosystem and the use of mangrove detritus as food source can explain the macro distribution of *S. cucullata* along the Kenyan coast.
The relative large discharges of fresh water in the coastal zone by rivers during the rainy season results in a serious drop in salinity. We will show that this drop in salinity triggers spawning. Our study also shows a downward movement of *S. cucullata* larvae shortly after fertilisation. This downward movement can be reversed by a sudden drop in salinity or by tidal movements.
Once the oyster larvae are in the water column and are in the immediate

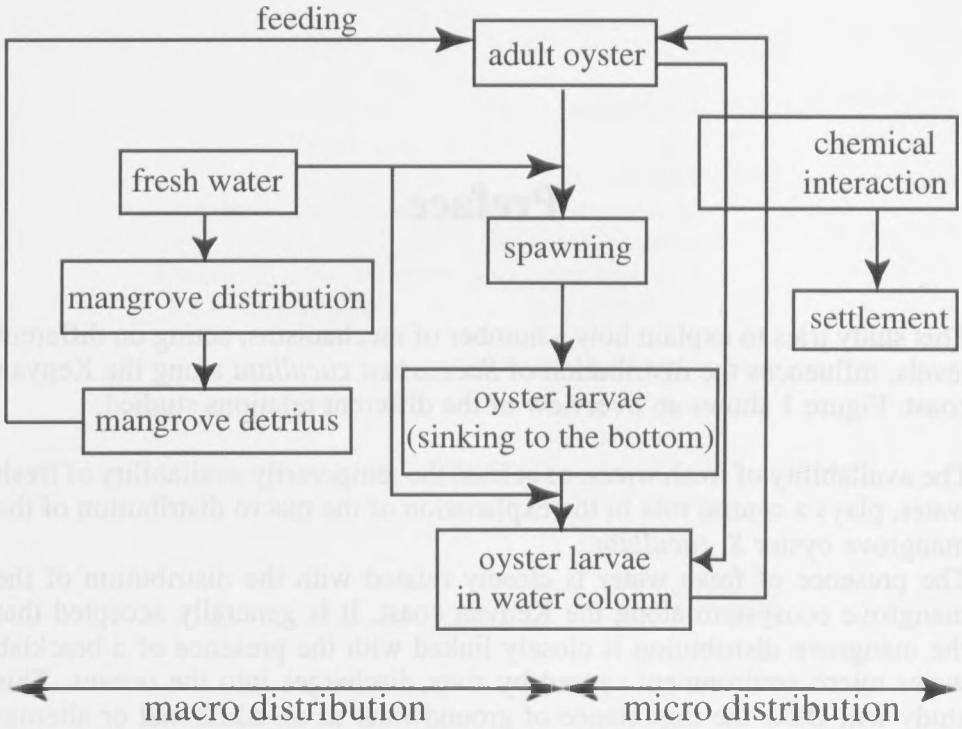
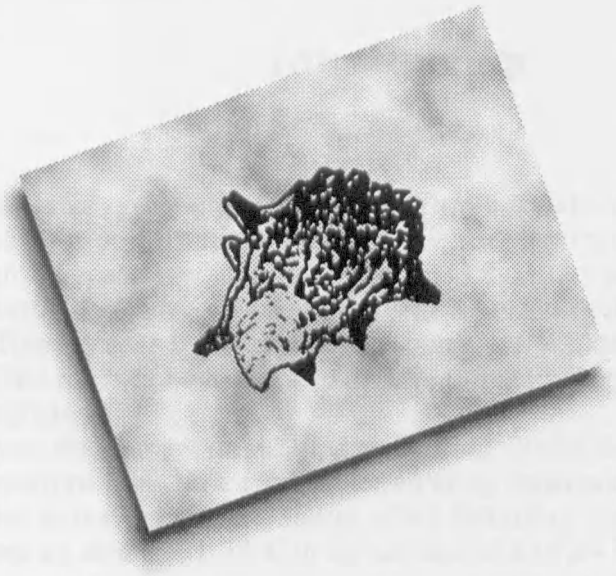


Figure 1. Overview of the different relations studied in this study.

neighbourhood of one or more adult oysters settlement is induced. This study will show that settlement is induced after multiple chemical signals between adult oysters and their larvae and vice versa. The chemical communication described in this study explains the micro distribution of *S. cucullata*.



Chapter

1

INTRODUCTION

Introduction

Mangrove trees grow in exposed areas. They are restricted to frost-free, tropical and subtropical climates, although their distribution is circumglobal. They grow on shallow, intertidal, protected mudflats wherever wave action is minimal. The tangle of stems and stilt roots slows down waves and currents. Therefore, mangrove-covered shores are the sites of depositions of muds. The muds are very dark and rich in organic matter derived from animals and plants.

Salinity in the mangrove swamps tends to be 20-38 ‰. A number of mangrove swamps have little river water entering them and have high evaporation rates so that salinity can rise to 40-42 ‰ during dry seasons. Water temperatures are around 31-34°C in the hot season and 20-30°C during the cooler rainy season.

Stenzel (1971) is one of the rare authors giving an overview of the ecology of mangrove oysters. They incrust the mangrove stems and stilt roots only in a narrow band up to 4 m wide at the edges of the swamps facing open water of tidal channels or of the centre of the lagoon (Van Someren and Whitehead, 1961). Edges of swamps facing prevailing winds have more oyster incrustations than the more protected edges. Oysters are absent in the centres of the swamps. Mud is the reason for these distributions of oyster incrustations. Wherever wave action is feeble, mud settles out on the mangrove, and oyster larvae avoid settling on mud-covered substrata. For the same reason oysters tend to colonise the undersurfaces of inclined mangrove stems rather than their top surfaces. The crotch on the underside of tripod-like mangrove stilts is commonly free of mud and is the favourite place for oysters to grow on.

Incrustations are restricted to between tides. Below low tide level the oysters remain covered by water continuously and therefore, remain exposed to such predators as crabs and fish. These predators are so numerous that they elim-

inate all young and thin-shelled oysters. Above average high tide level oysters become exposed to air and sunshine too long to survive; only barnacles can survive there. Periodic exposure of the intertidal oysters to air and sunshine has a strong influence on their growth habits. Oysters do not grow on mud bottoms except where such firm and not mud covered substrata as gastropod shells are available.

Many different oyster genera have invaded the mangrove biotope: *Crassostrea* through *C. rhizophorae* (Guilding, 1828) in the Caribbean and West Indies; *Lopha* through *L. folium* ecomorph *cristagalli* (Linné, 1758) in the region from the Indian Ocean to south-western Japan; and *Saccostrea* through *S. cucullata* (von Born, 1778) in tropical West Africa and East Africa to Honshu, Japan.

The various mangrove oysters have several ecomorphic features in common. They tend to produce thin fragile scalelike shell imbrications or shell shoots and delicate protruding frills. There is a tendency to clasping shell extensions which become auxiliary holdfasts. Many of the mangrove oysters have xenomorphic sculpture.

The delicate and thin-shelled features are caused by scarcity of free calcium ions and by abundance of planktonic food in these waters. Shell growth must be rapid, but material to build walls is scarce. In addition, the oysters must shut their valves during low tide for some time. During this period their blood tends to become more acid and must be buffered. This is done by some of the calcium carbonate of the shell (Stenzel, 1971). In other words, during low tide the shell wall not only quits increasing in thickness but must lose in thickness. Because wave action is minimal in mangrove swamps, delicate and thin-shelled features remain undamaged and are not greatly disadvantageous to the animal. In fact they may be advantageous in keeping enemies at a distance.

Saccostrea cucullata (von Born, 1778) is an edible oyster found in the upper littoral zone, following the terminology of Lewis (1964) and Hartnoll (1976). It is found on the trunks and the stilt roots of mangrove plants and rocky substrata in brackish marine environments. Day (1974) noted the zoogeographical distribution of this species in the western part of the Indian Ocean. Ecological studies on this species have been carried out over the entire region: Seychelles (Taylor, 1968), Aldabra (Taylor, 1970), Tanzania (Hartnoll, 1976), Somalia (Chelazzi and Vannini, 1980) and Kenya (Ruwa, 1984; Okemwa *et al.,* 1986).

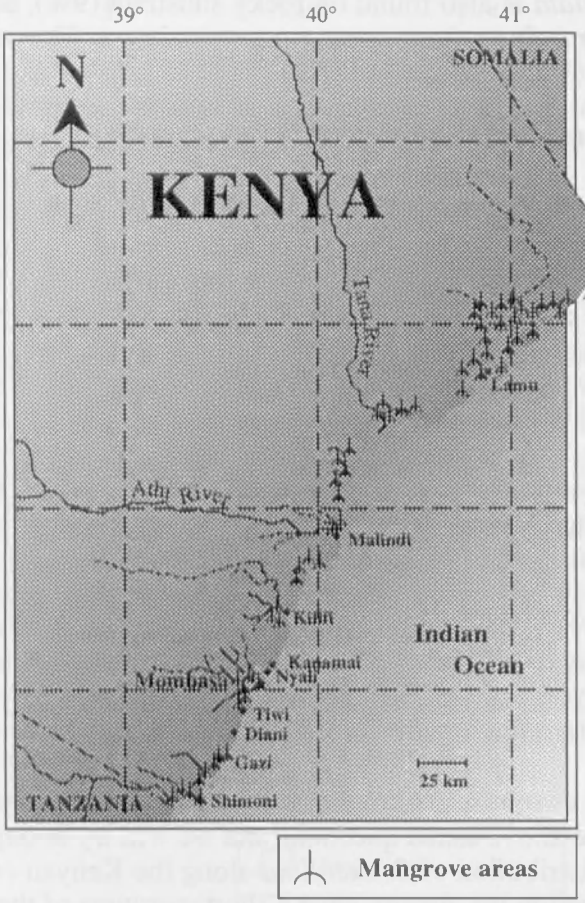


Figure 1. Distribution of the mangrove forest in Kenya.

Figure 1 shows the distribution of mangrove forests along the Kenyan coast. Figure 2 shows the distribution of *S. cucullata* in the same region. Comparing both figures we can see a clear relation between the distribution of the mangrove forest and the mangrove oyster *S. cucullata*. It is clear that *S. cucullata* did not steel its nickname of 'mangrove oyster'. A majority

(87%) of all *S. cucullata* is found on the trunks and stilt roots of mangrove trees. *S. cucullata* is also found on rocky substrata (9%), but always within a range of 5 km from the nearest mangrove forest. The remaining 4% are specimens growing on other substrates available in the tidal zone. One of the largest beds of *S. cucullata* is that found on the reef extending from Ras Ngomeni, north of Malindi (Kenya). But even in this case the mangrove forest is near.

To explain the 'macro' distribution of *S. cucullata* we have to find the link between the distribution of mangroves and mangrove oysters. A first link is the use of mangrove trunks and stilt roots as substrate for *S. cucullata*. However, this does not explain why *S. cucullata* is found on rocks and other useful substrates in the neighbourhood of mangrove forests and not on the same substrates when those are found further away from the mangrove environment. Does the mangrove ecosystem play a crucial role in the life cycle of *S. cucullata*? As food source perhaps? Or do we have to find a common variable regulating both mangrove forest and *S. cucullata* distribution?

Walking through a mangrove forest in Kenya and studying the 'micro' distribution of the mangrove oyster, one thing becomes clear on a very short notice. There are two possibilities: a mangrove tree full of mangrove oysters or no mangrove oysters at all on a tree. *S. cucullata* larvae spend approximately 28 days in the water column. During this period the larvae are dispersed over large parts of the mangrove forest. How do they find each other? How do they find that onetree where adult oysters settled one or more years before?

In the next 4 chapters, containing 9 scientific articles, we will try to find answers on the above asked questions and we will try to explain the 'macro' and 'micro' distribution of *S. cucullata* along the Kenyan coast.

In this introduction we already gave a short overview of the ecology and the 'macro' distribution of *S. cucullata* along the Kenyan coast. In chapter 2 we go more deeply into the ecology of *S. cucullata*. We also related the 'micro' distribution of *S. cucullata* to variations in shell form and shell length. The second article of chapter 2 contains additional information on the correct use of the name *S. cucullata* and gives a description of the shell form, making use of univalent and multivalent variables. The second article was awarded the Mac Leod prize 1991 by the Belgian Academy for Science, Letters and Fine Arts. For those reasons we preferred to give the full text of both articles.

Walsh (1974) suggested the existence of extensive mangal depended upon

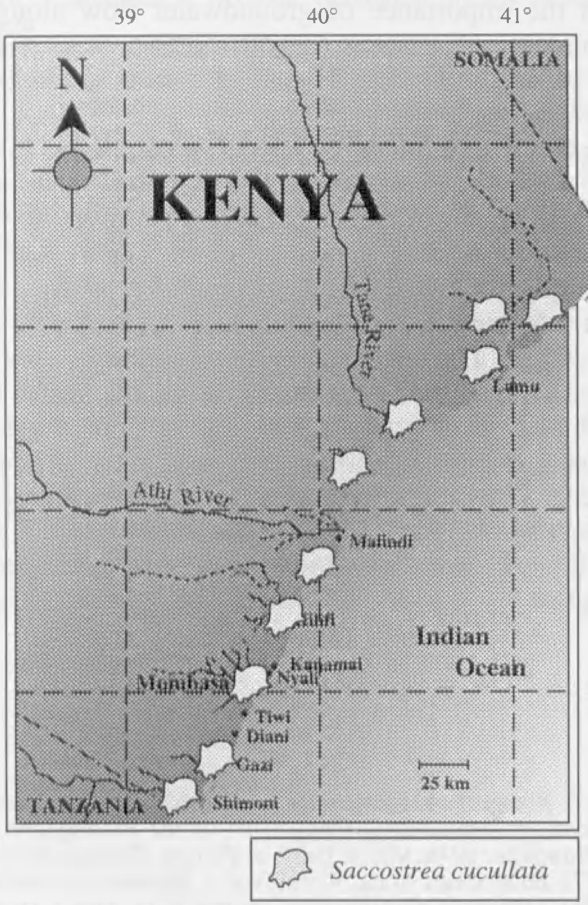


Figure 2. Distribution of the mangrove oyster *Saccostrea cucullata* in Kenya.

five basic factors. Chapman (1975, 1977, 1984) believed there are seven: (1) air temperature, (2) ocean currents, (3) protection from wave action, (4) shallow shores, (5) salt water, (6) tidal range, and (7) substrate. In chapter 3 we will show that at least one more variable has to be added to this list: the presence of fresh water. There is a consensus that the brackish water micro envi-

ronment of mangrove areas is caused by river discharges into the ocean.
Chapter 3 shows the importance of groundwater flow along the Kenyan coast and its influence on the mangrove distribution. A second contribution in this chapter studies the influence of groundwater on a number of ecological variables of mangrove trees.
Chapter 4 describes the feeding behaviour of *S. cucullata*. It will stress the importance of dissolved organic carbon (DOC) as food source for the mangrove oyster. We will try to find the original source of the DOC component, taken up by *S. cucullata*, and try to relate it to its 'macro' distribution.
The last chapter studies the settling behaviour of *S. cucullata* larvae. A first contribution to this chapter deals with the spawning behaviour and the larval culture of *S. cucullata*. The second contribution shows how one or more chemical inducers mediate the settlement of *S. cucullata* larvae, explaining the 'micro' distribution of the species. The aggregations of adult *S. cucullata*, found in the mangrove forest, influence the settling behaviour and the survival of their larvae. This is described in a last contribution of chapter 5.
The epilogue combines the conclusions of the different articles presented in this Ph.D. study. It shows how behavioural aspects of the mangrove oyster *S. cucullata* can explain its 'macro' and 'micro' distribution along the Kenyan coast.

References

Chapman, V. J. (1975). Mangrove biogeography. In: Walsh, G. D., Snedaker, S. C., Teas, H. J. (Ed.) Proceedings International Symposium on the Biology and Management of Mangroves, Honolulu, 1974. Vol. 1. Univ. of Florida, Gainesville, p. 3-22

Chapman, V. J. (1977). Ecosystems of the World. Vol. 1. Elsevier, Amsterdam

Chapman, V. J. (1984). Mangrove biogeography. In: Por, F. D., Dor, I. (Ed.) Hydrobiology of the Mangal. Dr. W. Junk Publishers, The Hague, p. 15-24

Chelazzi, G., Vannini, M. (1980). Zonation of intertidal molluscs on rocky shores of southern Somali. Estuar. coast. mar. Sci. 10: 569-583

Day, J. H. (1974). A guide to marine life of South African shores. Balkema, Cape Town

Hartnoll, R. G. (1976). The ecology of some rocky shores in tropical East Africa. Estuar. coast. mar. Sci. 4: 1-21

Lewis, J. R. (1964). The ecology of rocky shores. Hodder & Stoughton, London

Okemwa, E. R., Ruwa, R. K., Polk, P. (1986). The autecology of the edible oyster *Crassostrea cucullata* Born, 1778: size-related vertical distribution at Mkomani, Mombasa. J. Sci. Ser. B 7: 9-14

Ruwa, R. K. (1984). Invertebrate faunal zonation on rocky shores around Mombasa, Kenya. Kenya J. Sci. Ser. B 5: 49-65

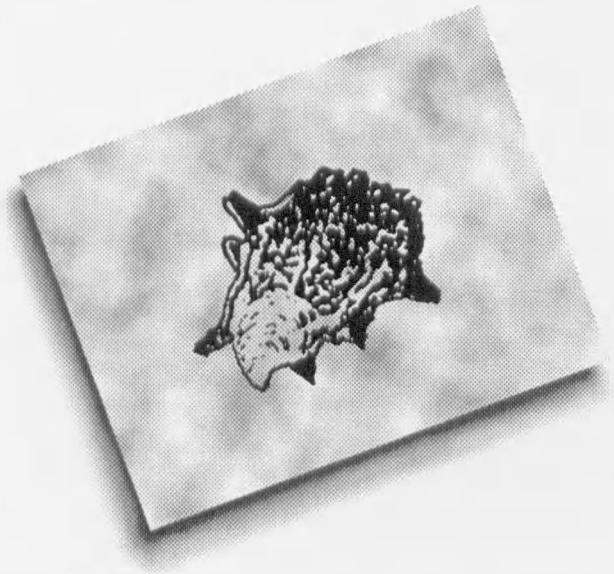
Stenzel, H. B. (1971). Oysters. In: Moore, R. C. (Ed.) Treatise on Invertebrate Paleontology. Part N. Vol. 3. Mollusca 6. Bivalvia. Geological Society of America, Boulder, p. N953-N1224

Taylor, J. D. (1968). Coral reef associated invertebrate communities (mainly molluscan) around Mahe, Seychelles. Phil. Trans. r. Soc., Lond. Ser. B 254: 129-206

Taylor, J. D. (1970). Intertidal zonation at Aldabra atoll. Phil. Trans. r. Soc., Lond. Ser. B 260: 173-213

Van Someren, V. D., Whitehead, P. J. (1961). An Investigation of the Biology and Culture of an East African Oyster *Crassostrae cucullata*. Col. Off. Fish. Publ. 14: 2-41

Walsh, G. E. (1974). Mangroves: a review. In: Reinhold, R., Queen, W. (Ed.) Ecology of Halophytes. Academic press, New York, p. 51-74



Chapter

2

ECOMORPHOLOGY

Ecomorphology of the mangrove oyster *Saccostrea cucullata* (Born, 1778)

Abstract

Variations of shell form and shell length were studied for oysters growing in the mangroves of Gazi Creek, Kenya, and related to different environmental factors.

For the study of the form, Fourier analysis was performed on the circumference of 85 oysters. The resulting coefficients were compared among specimens using cluster analysis. The correspondence between this classification and substrate diameter is virtually perfect (only one misclassification out of 85 oysters). For the clusters based on height above chart datum, 9 specimens, all on intermediate height levels, were misclassified. Orientation with respect to tidal current had 13 misclassifications. Mangrove species seemed to influence form only marginally, if at all.

In the study of the size of *Saccostrea cucullata*, the length of 956 oysters, growing along two transects were measured, and correlated with several environmental factors. Oyster length was not related to substrate diameter or its orientation with respect to the main current. Length was not influenced by density up to a cover of 70%. For densities higher than 70%, there was a fairly strong negative correlation (r^2=0.634, n=217). Length was not correlated with height above bottom (base of the tree) for heights lower than 20 cm, while oysters growing closer to the bottom were smaller. The correlation with height above chart datum was negative but very low (r^2=0.060, n=957). However, if all measurements of oysters closer than 20 cm to the bottom, and all from a density of more than 70% cover are deleted from the data set, the correlation with height increased dramatically, the slope still being negative (r^2=0.859, n=543).

Introduction

Saccostrea cucullata (Born, 1778) is an edible oyster found in the upper littoral zone, following the terminology of Lewis (1964) and Hartnoll (1976). It is found on the trunks and the stilt roots of mangrove plants and rocky substrata in brackish marine environments. Day (1974) noted the zoogeographical distribution of this species in the western part of the Indian Ocean. Ecological studies of this species have been carried out over the entire region: Seychelles (Taylor, 1968), Aldabra (Taylor, 1970), Tanzania (Hartnoll, 1976), Somalia (Chelazzi & Vannini, 1980) and Kenya (Ruwa, 1984; Okemwa *et al.*, 1986).

Previous research already suggested a relationship between the form of this oyster and the substrate. Kesteven (1941) stated that the form of the oyster is partly determined by the form of the substrate. Van Someren & Whitehead (1961) distinguished different categories of form of oysters, and correlated these forms with different types of habitats, and with different types of substrate.

These observations were however, qualitative. The first quantitative work was done by the above authors. They compared oysters growing on different artificial substrates by Fourier analysis. Their conclusion was that the diameter of the substrate has an influence on the form of the shell of *Saccostrea cucullata*.

Various studies show intraspecific differences in size of molluscs in relation to environmental parameters (Comfort, 1946; Ebling *et al.*, 1962; Vermeij, 1972, 1973; Ruwa & Brakel, 1981). Okemwa *et al.* (1986) showed that such differences also exist in the mangrove oyster *Saccostrea cucullata*, growing on a rocky substratum. The present investigation was undertaken to study the effects of different environmental factors on the size of this oyster growing in a mangrove ecosystem. The results of this study may be of immediate importance to the culture of *Saccostrea cucullata*.

Material and methods

Observations were made between July and September 1989 in a small creek near Gazi, a small village 49 km south of Mombasa (Kenya). Seven species

of mangrove trees occur at Gazi (Gallin *et al.*, 1989): *Avicennia marina* (Forsk.) Vierh., *Bruguiera gymnorrhiza* (L.) Lam., *Ceriops tagal* (Perrotet) C. B. Robinson, *Lumnitzera racemosa* Willd., *Rhizophora mucronata* Lam., *Sonneratia alba* J. Smith and *Xylocarpus granatum* Koenig. An elaborate description of this creek can be found in Tack (1990).

The environmental factors

Orientation with respect to the main tidal current was taken as either perpendicular or parallel. Perpendicular was further subdivided into facing the inflowing current or facing the outflowing current. The diameter of the branch serving as a substrate was measured with calipers to the nearest mm. The height above bottom was measured to the nearest cm.
To measure approximate density, we made vertical transects on each of the 10 cm vertical bands. These transects were made in four directions (facing North, East, South and West). The approximate density was expressed as the percentage of the vertical transect that was covered by oysters.
Height above chart datum was determined following the method of Okemwa *et al.* (1986). In this method, the highest level the water reaches during the tidal cycle is marked. The height of this level is taken to be the same as the one given in the tide table for the same tidal cycle, and used as an absolute reference for the rest of the measurements. From this reference, height above chart datum was determined to the nearest cm.

Shell form

Sampling

85 oysters were collected in a restricted area of 10 x 10 m. This area was chosen for the availability of all environmental conditions of interest. 'Collection' was done by making a photograph of the oyster. These photographs were taken so that the oyster filled only 1/9 of the surface of the image, to avoid distortion.
The sample taken was stratified with respect to environmental parameters. Only oysters that were growing isolated (i.e. not touching any other oyster) were included in the sample. In this way, crowding did not interfere with the

analysis: all oysters included in the sample had the opportunity to grow to their full size.
The environmental parameters studied were (1) species of the mangrove tree serving as a substrate, (2) diameter of the branch or pneumatophore on which the oyster was growing, (3) height above chart datum and (4) orientation with respect to the main tidal current.
Four species of mangrove trees were present in the sampling site. The number of oysters taken from these species were more or less equal: 21 from *A. marina*, 22 from *B. gymnorrhiza*, 23 from *R. mucronata* and 19 from *S. alba*. 28 oysters were growing parallel with the current, 29 were facing the inflowing current, 28 the outflowing current.
The diameter of the substrate, a continuous variate, was dichotomised by taking oysters only growing either on small branches (less than 30 mm diameter) or large branches (more than 100 mm diameter). The sample included 58 oysters of small branches, and 27 oysters of large branches.
Height above chart datum varied between 2.20 and 3.40 m; all heights are represented in the sample (Fig. 1). It was not possible to dichotomise this variate, as was done for the diameter of the substrate. Not enough oysters were present with the right combination of environmental parameters.
The photographs of the oysters were magnified 10 times, and redrawn on paper. These drawings were digitised using a Complot series 7000 digitiser, coupled to an IBM AT. The resolution of the digitiser was 0.025 mm; points were taken at intervals of 0.8 mm around the perimeter. The average length of the digitised circumference was about 1 m.

Fourier analysis
The form of closed contours can be quantified using Fourier analysis, by considering the polar coordinates of the points of the contour, with the origin of the polar coordinates chosen within the contour (e.g. Anstey & Delmet, 1972, 1973; Christophers & Waters, 1974; Ehrlich & Weinberg, 1970; Healy-Williams & Williams, 1981; Kaesler & Waters, 1972; Younker & Ehrlich, 1977). This method was also used to describe the form of oysters by the authors.
A limitation of the method involving polar coordinates is that only those forms where there is only one intersection between the boundary and a ray from the origin of the polar coordinates can be analysed. The form of most of the oysters is quite irregular, and does not satisfy this condition. Hence

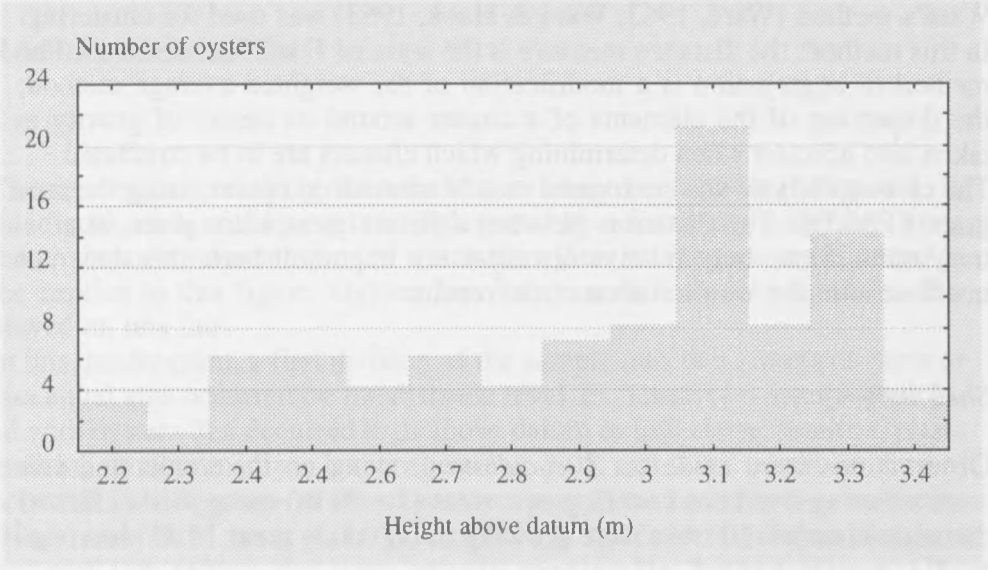


Figure 1. Number of oysters as a function of height above datum (m).

another method, the elliptical method, has been used in this study. For this method, both x and y coordinates of the boundary are interpreted as a function of distance along the boundary. The two resulting functions are periodical, with a period given by the length of the boundary, and can be separately analysed using the Fourier transform (Ferson *et al.,* 1985; Kuhl & Giardini, 1982).

The analysis was performed on an IBM AT. Before performing the transformation, a total of 64 equally spaced points are interpolated between the digitised points. For each of the forms to be analysed, 32 harmonics are calculated. Preliminary tests showed that, with this number of harmonics, all features of the form of the circumference could be described accurately.

Cluster analysis

The coefficients resulting from the Fourier transform were entered in a cluster analysis, to detect groups of oysters of similar form.

Ward's method (Ward, 1963; Ward & Hook, 1963) was used for clustering. In this method, the distance measure is the squared Euclidean distance. The method of aggregation is a modification of the weighted average method; the dispersion of the elements of a cluster around its centre of gravity is taken into account when determining which clusters are to be combined.
The cluster analysis was performed on a Macintosh computer, using the program SPSS/PC. The distances between different groups are given in arbitrary units. Since only relative distances are important here, this does not interfere with the interpretation of the results.

Shell length

Observations were made on *S. cucullata* growing on the trunks and stilt roots of mangrove trees covering two areas (4 x 20 m) on opposite sides of the main channel. 20 trees were growing in our study area: 12 *R. mucronata*, 2 *A. marina* and 6 *S. alba*.
Each trunk and stilt root was divided into consecutive 10 cm vertical bands starting from the bottom of the tree trunk. ln each vertical band we collected, if present, an oyster growing on the northern, the eastern, the southern and the western side of the trunk or stilt root.
The shell length of all collected oysters was measured with calipers to the nearest 0.1 mm. Length was defined as the distance from the centre of the hinge to the farthest point of the opposite end. For each of the measurements, a number of environmental variables were recorded: (1) species of the mangrove tree and diameter of the branch serving as a substrate, (2) approximate density of the oysters, (3) height above the bottom (base of the tree trunk), (4) height above chart datum, and (5) orientation with respect to the main tidal current.
A regression analysis was performed to try to correlate length with each of these variables separately. These variables were taken as the main factors to study in relation to oyster length.

Results and discussion

Shellform

The dendrogram resulting from the cluster analysis is shown in Figure 2. In this figure, each of the oysters is represented by a four-letter code, indicating the environmental conditions in which the oyster was found, as explained in the caption to this figure. Oysters less than one unit distance apart are displayed on one line.

In this dendrogram, a first division of the sample into two groups of more or less equal size occurred on the distance level 25. These two groups coincided with oysters that occurred high above datum or low above datum. Oysters growing in intermediate levels (between 3.05 and 3.13 m above datum) occurred in both groups of the cluster analysis. If the limit for discriminating 'High' and 'Low' is set at 3.07 for oysters in the field, 9 misclassifications occurred (i.e. oysters that were actually growing low in the tidal zone, but were classified with the oysters growing high in the tidal zone by the cluster analysis). A contingency table was constructed, where one of the nominal variables is the actual characteristic of the specimen, and the other the predicted characteristic based on the membership to a group in the cluster analysis. This procedure permitted the calculation of a Chi square value, indicating the significance of the association between observed and predicted characteristics. The Chi square value and its probability are given in Table 1.

Table 1 also gives the value for Lambda, Goodman and Kruskal's measure of predictive ability (Everitt, 1977). This coefficient is a measure of association between two nominal variables, and ranges from 0 to 1 (0 indicating no association, 1 a perfect association). The significance of Lambda is the same as the significance of the contingency table on which it is based.

Each of the two above mentioned groups were separated into two subgroups, on distance levels of 9 and 14 respectively. These two divisions coincided with the oysters growing on small or on large diameters of substrate. Only one misclassification occurred for this criterion. Statistics for this division (Chi square, p and Lambda) are also given in Table 1.

The interpretation for the two other environmental parameters was not as straightforward, in the sense that there was no strict dichotomy subdividing each of the groups on levels lower than the ones discussed above. However,

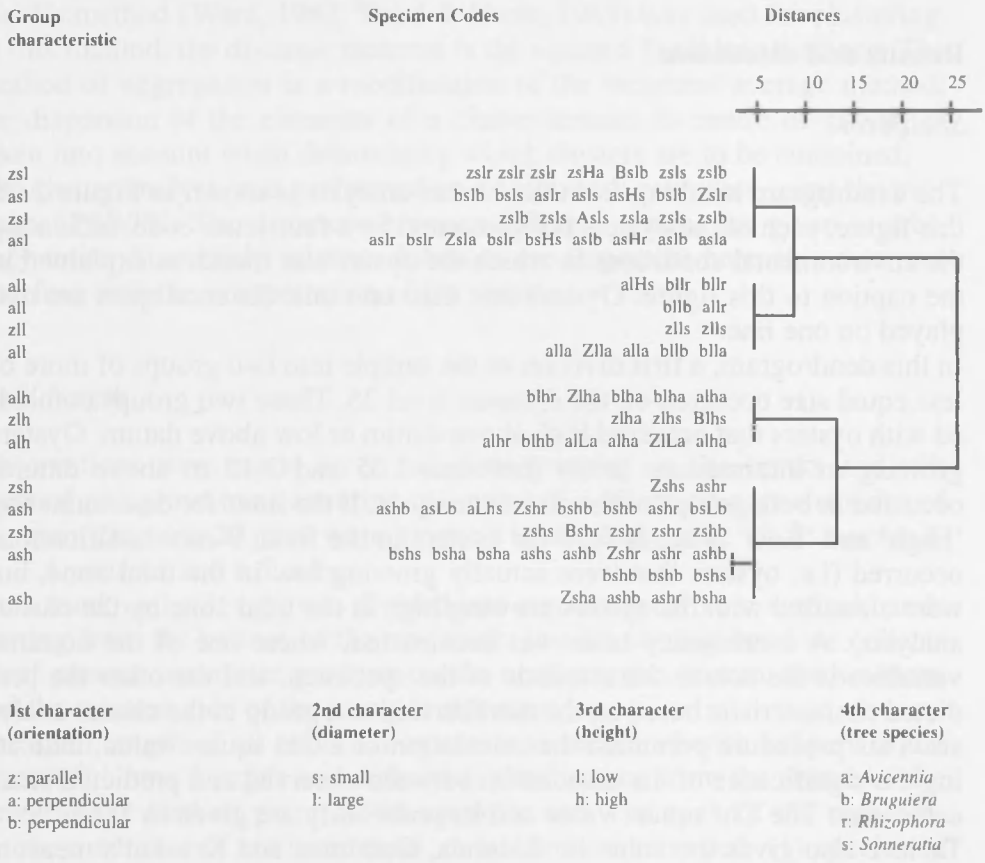


Figure 2. Results of cluster analysis. Distance units are arbitrary. Each four-character code represents one oyster. Group characteristics were determined on the basis of majority. a and b are considered together, and written as 'a' in the group characteristics. Upper case characters are misclassifications (not for tree species). Oysters on one line are less than 1 unit distance apart.

it was still possible to assign a group characteristic to each of the groups of the cluster analysis with respect to orientation, if only two categories are considered (parallel or perpendicular). If the group characteristic is taken to be the value of the majority in the group, 13 misclassifications occurred. As can be seen from Table 1, this is still a highly significant result, though the value for the index of predictive ability was much lower than in the two cases

Environmental parameter	Chi square	*p*	Lambda
Height above datum	49.69	<0.001	0.84
Substrate diameter	75.99	<0.001	0.96
Orientation	31.69	<0.001	0.49
Mangrove species	64.6	<0.029	0.21
Avicennia or not	34.0	<0.003	0.11

Table 1. Statistics of the number of misclassifications. The first three tests are based on a 2 x 2 table of predicted and actual group membership. The last two lines are based on respectively a 16 x 4 and a 16 x 2 table of frequency of occurrence of the different values for the tree species in the 16 different groups of Figure 2.

discussed above. The two values for perpendicular (facing outflowing or facing inflowing current) seemed to have no influence whatsoever: these values occurred together in most of the groups.

For the last environmental parameter, the species of the mangrove tree, no group characteristic could be determined, so it was not possible to construct a contingency table as before. If the randomness of the distribution of the actual value for the mangrove species over the different groups is tested using a 4 x 16 table, the value of Chi square turns out to be just significant. However, in view of the fact that most of the expected frequencies are very low, not too much importance should be attached to this value.

Oyster form is non-randomly distributed with regard to *Avicennia*, i.e., if a group of related forms contained an oyster growing on *Avicennia*, the other oysters of the group had a higher probability of having been collected on *Avicennia* as well. This can be tested by constructing a 2 x 16 contingency table, where the two categories are respectively non-*Avicennia* and *Avicennia*. The Chi square value for this table is highly significant.

It is clear from these results that the form of the oysters is influenced by both height above datum and diameter of the substrate. Height above datum had the most important influence, as reflected in the fact that the two groups were separated at the highest level of distance. Differences of form resulting from differences of diameter of the substrate were smaller. The number of mis-

classifications for this last environmental parameter was less in this study, but this was caused by the way in which the sampling was done (continuous sampling throughout the range for height above datum, versus sampling on the extremes for substrate diameter).
The influence of substrate diameter was already demonstrated by a preliminary study, using analogue methods on a small sample of oysters growing on artificial substrates. The influence of the height above datum on size was demonstrated earlier (Okemwa *et al.,* 1986), but no discussion of the influence of this parameter on the form had been described up to now.
Orientation with respect to main tidal current seemed to have a weaker, but still highly significant influence on the form. The species of mangrove tree had no apparent effect on the form of the oysters, if the four species were

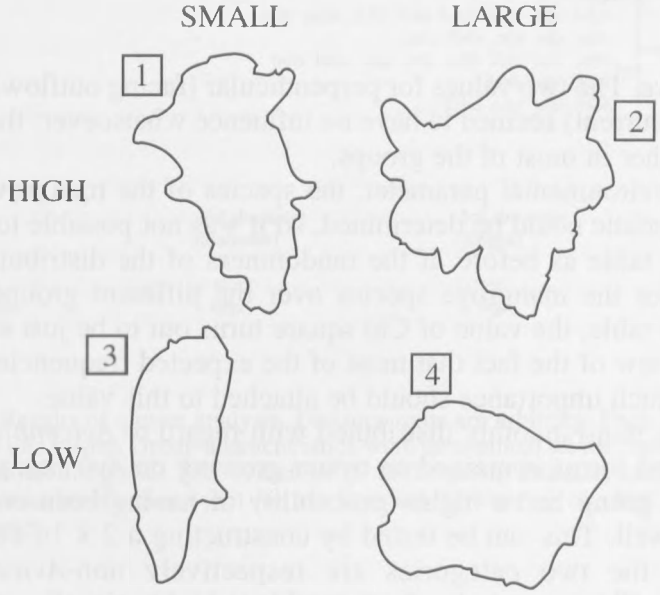


Figure 3. Outlines of shells of oysters growing in different environmental conditions. 1: small diameter substrate, high above datum; 2: large diameter substrate, high above datum; 3: small diameter substrate, low above datum; 4: large diameter substrate, low above datum.

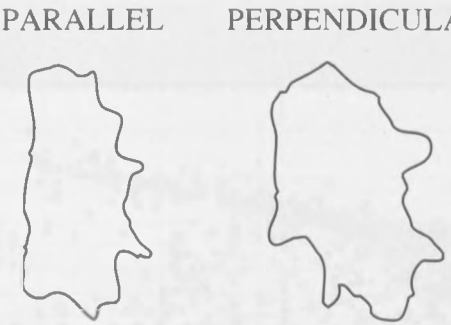


Figure 4. Outlines of shells of oysters growing in different environmental conditions. Left: parallel to tidal current; right: perpendicular to tidal current.

taken separately. If *Avicennia* was considered separately, the distribution over the different groups of the cluster analysis was clearly non-random. This would imply a different form for the oysters growing on *Avicennia*, as compared to the other species. This does not come as a complete surprise: the density of oysters was much lower on this species of mangrove tree. The mechanism of this phenomenon remains to be investigated, but it is not at all impossible that the same factor would also influence the form of the oysters. This factor could be a mild oyster repellent, or the texture of the bark of *Avicennia*, that is smoother than that of the other mangrove species.

Shell length

The diameter of the branch serving as substrate and the orientation with respect to the main tidal current appeared to be of no importance for shell length. Shell length was not related to density up to a cover of 70% . For densities higher than 70% there was a fairly strong negative correlation (r^2=0.634, n=217), as one would expect (Fig. 5).
Length does not seem to be correlated with height above bottom for heights higher than 20 cm. At a height lower than 20 cm above the bottom, the oysters are clearly shorter than at higher levels.
The relationship between shell length of *S. cucullata* and the height above chart datum is shown in Figure 6. From this figure it is clear that when shell

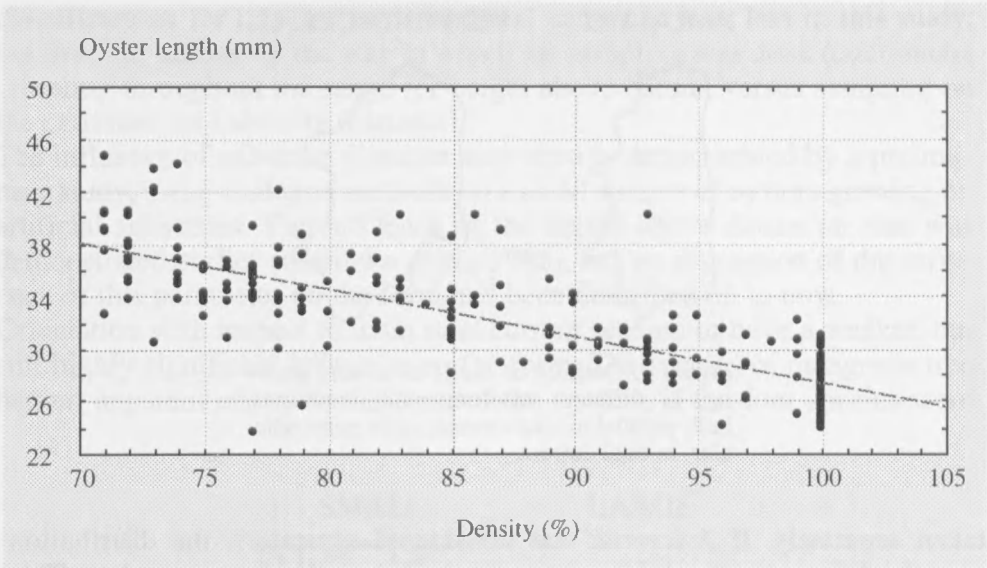


Figure 5. Relationship between density (% cover) and oyster length (mm) for densities higher than 70% (r^2=0.634; n=217).

length is plotted against height above datum the y values are not independently and normally distributed for any given value of x. However, according to Ricker (1973) regression analysis is very robust to deviations from its underlying assumptions. Noting the fact that we have to interpret the following results with the necessary caution we can still calculate the regression parameters. The correlation between oyster length and height above chart datum is negative and very low (r^2=0.060; n=957). If all measurements of oysters closer than 20 cm to the bottom, and all from a density of more than 70% cover are deleted from the data set, the negative correlation increased dramatically (r^2=0.850; n=543; Fig. 7).

The fairly strong negative correlation coefficients (r^2) demonstrate the influence of density on oyster length for densities higher than 70% cover. Other authors have demonstrated that density may affect growth rate and size in some littoral bivalves (Trevaillon *et al.,* 1970). This relationship (between size and density) is not found by Okemwa *et al.* (1986). They measured the shell length of *Saccostrea cucullata* on a rocky substratum and concluded

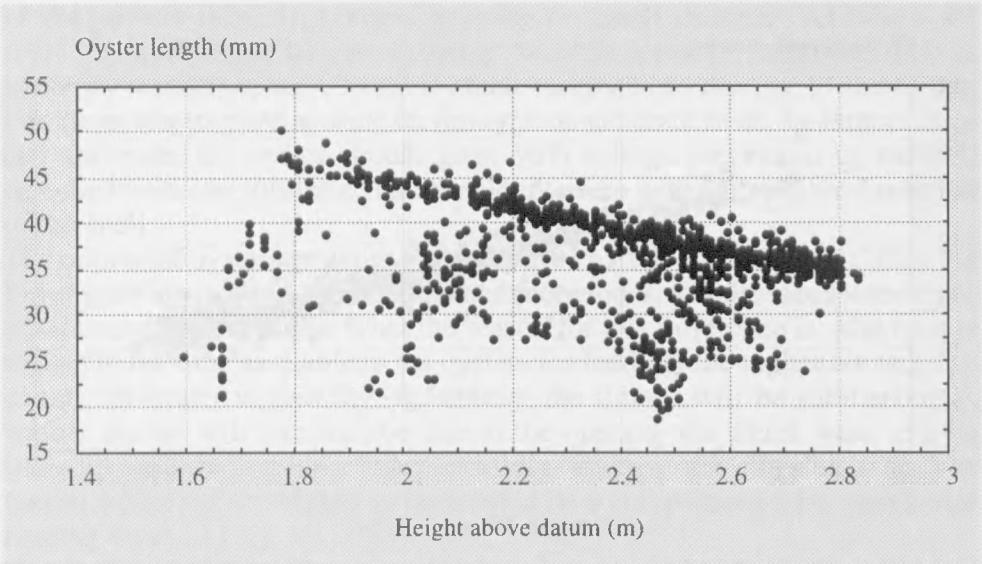


Figure 6. Relationship between height above chart datum (m) and oyster length (mm). All measurements are included in this figure.

that size is only a function of height above chart datum. However, in Okemwa *et al.*'s study, oyster density was smaller than 70% cover throughout. Combined with our results, this indicates that oyster density does not affect oyster size for densities lower than 70% cover.

Shell lengths of *S. cucullata* are small at a height lower than 20 cm above the bottom as compared to oysters growing higher. This may be due to the higher sediment load closer to the muddy bottom. Because oysters are filter feeders, they may have to expend more energy in filtering the water at lower levels to get an equal amount of food as do the oysters growing at higher levels. The correlation between oyster length and the height above datum is negative and very low. However, when we removed all oysters growing lower than 20 cm above the bottom, and all oysters growing on substrata with density greater than 70% cover, the correlation becomes very strong (r^2=0.850; n=543). This high negative correlation coefficient demonstrates that *Saccostrea cucullata* exhibits size-related patterns in its vertical distribution with an up-shore reduction in shell length.

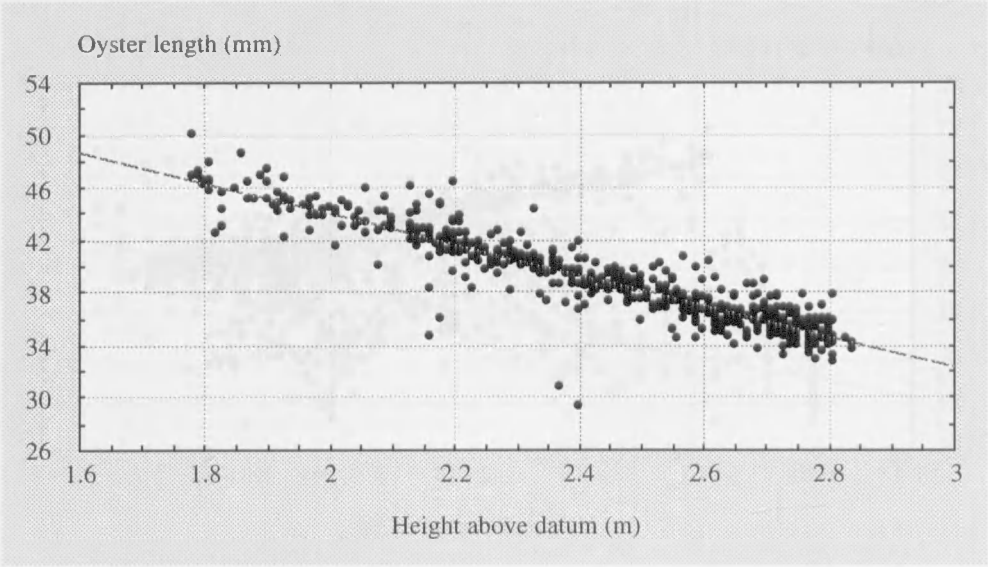


Figure 7. Relationship between height above chart datum (m) and oyster length (mm) (r^2=0.850; n=543). Measurements on oysters growing lower than 20 cm above the bottom and/or in areas with an oyster density higher than 70% cover are excluded from this data set.

Conclusions

The form of the oysters is clearly related to both height above datum and diameter of the substrate. Changes of form associated with differences in height above chart datum were the most important, as reflected in the fact that the two groups were separated at the highest level of distance. Differences of form resulting from differences of diameter were smaller. The number of misclassifications for this last environmental factor was less in this study, but this can be attributed to the way in which the sampling was done (continuous sampling for height above datum, as opposed to dichotomised sampling for substrate diameter).

The distinction between these four groups can be related to the appearance

of the oysters (Fig. 3). Oysters growing on small diameter substrates are more elongated than the ones growing on large diameter substrates. This is what one would expect: 30 mm is small compared to the size of the oyster. For the oyster to grow as wide on this type of substrate as on the larger diameter substrate, the oyster should grow with a large proportion of its shell detached from the substrate. This would interfere with the structural strength of the shell.

The outline of oysters growing higher above chart datum is 'wavier' than the lower growing oysters: their outline had more lobes, and the lobes were more pronounced. It is not clear what the reason for this difference is. Maybe it is related to the time available to the oysters for feeding: the higher above chart datum the oyster is growing, the shorter the time it will be submerged. A wavier outline will increase the size of the opening via which water can be taken in or expelled, and hence possibly increase the filtering rate. The wavier outline of the higher oysters could be a compensation for the shorter feeding time.

Orientation with respect to main tidal current seemed to have a weaker, but still highly significant correlation with form. Once again, this distinction is also apparent from a visual inspection of the outline of the oysters (Fig. 4). Oysters growing perpendicular to the tidal current often have lobes all around their outline, as opposed to oysters growing parallel to the current, that have lobes mainly on one side.

Height above chart datum, and thus percentage of time immersed, seems to be the primary factor determining the shell length of the oysters. This relationship is obscured by the influence of crowding and proximity to the bottom. Competition for space is the obvious mechanism by which crowding can influence the length of the oyster shell. The way in which proximity to the bottom is influencing oyster growth remains to be investigated, but a probable mechanism is the interference of high sediment load with the filter feeding of the oysters.

As with the correlation between form and height above chart datum, it seems logical to look for an explanation of the correlation between shell length and height in the time an oyster is submerged, and hence feeding time. Oysters growing lower have a longer proportion of the day available for feeding, hence can be expected to grow faster. If the maximum size an oyster can reach is dictated by age, rather than by size itself, an upshore reduction in size is the expected trend.

This study has only demonstrated the existence of the relationship between environmental parameters on one hand, and form and size of the oysters on the other. A following step in this research should be the construction of a functional model, to investigate the causal relationship between morphology and the environmental parameters, and to investigate the hypotheses formulated above.

Acknowledgements

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References

Anstey, R. L. & D. A. Delmet, 1972. Genetic meaning of zooecial chamber shapes. Science 177: 1000-1002.

Anstey, R. L. & D. A. Delmet, 1973. Fourier analysis of zooecial shapes in fossil tubular bryozoans. Geol. Soc. Am. Bull. 84: 1753-1764.

Chelazzi, G. & M. Vannini, 1980. Zonation of intertidal molluscs on rocky shores of southern Somali. Estuar. coast. mar. Sci. 10: 569-583.

Christopher, R. A. & J. A. Waters, 1974. Fourier series as a quantitative descriptor of miospore shape. J. Paleontol. 48: 697-709.

Comfort, A., 1946. *Patella vulgata* L. relation of habitat to form. J. Conch. 22: 216-217.

Day, J. H., 1974. A guide to marine life of South African shores. Balkema. Cape Town, 300 pp.

Ebling, F. J., J. F. Sloane & H. M. Davies, 1962. The ecology of Lough Ine. XII. The distribution and characteristics of *Patella* species. J. anim. Ecol. 31: 457-470.

Ehrlich, R. & B. Weinberg, 1970. An exact method for characterisation of grain shape. J. Sed. Petrol. 40: 205-212.

Everitt, B . S ., 1977. The analysis of contingency tables. Chapman and Hall. London, 128 pp.

Ferson, S., F. J. Rohlf & R. K. Koehn,1985. Measuring shape variation of twodimensional outlines. Syst. Zool. 34: 59-68.

Gallin, E., E. CoppeJans & H. Beeckman, 1989. The mangrove vegetation of Gazi Bay (Kenya). Bull. r. Soc. Belg. 122: 197-207.

Hartnoll, R. G., 1976. The ecology of some rocky shores in tropical East Africa. Estuar. coast. mar. Sci. 4: 1-21.

Healy-Williams, N. & D. F. Williams, 1981. Fourier analysis of test shape of planktonic

foraminifera. Nature 289: 485-487.
Kaesler, R. L. & J. A. Waters, 1972. Fourier analysis of the ostracode margin. Geol. Soc. Am. Bull. 83: 1169-1178.
Kesteven, G. L., 1941. The biology and cultivation of oysters in Australia. I. Some economic aspects. Commonwealth of Australia. Melbourne, 32 pp.
Kuhl, F. P. & C. R. Giardini, 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing 18: 236-258.
Lewis, J. R., 1964. The ecology of rocky shores. Hodder & Stoughton, London, 323 pp.
Okemwa, E. R., R. K. Ruwa & P. Polk, 1986. The autecology of the edible oyster *Crassostrea cucullata* Born,1778: sizerelated vertical distribution at Mkomani, Mombasa. Kenya J. Sci. Ser. B 7: 9-14.
Ricker, W. E., 1973. Linear regressions in fishery research. J. Fish. Res. Bd Can. 30: 409-434.
Ruwa, R. K., 1984. Invertebrate faunal zonation on rocky shores around Mombasa, Kenya. Kenya J. Sci. Ser. B 5: 49-65.
Ruwa, R. K. & W. H. Brakel, 1981. Tidal periodicity and size-related variations in the zonation of the gastropod *Nerita plicata* on East African rocky shore. Kenya J. Sci. Ser. B 2: 61-67.
Tack, J. F., 1990. Ecomorfologie van de mangroveoester *Crassostrea cucullata* (Born, 1778). Unpublished M. Sc. thesis, Free university of Brussels, 99 pp.
Taylor, J. D., 1968. Coral reef associated invertebrate communities (mainly molluscan) around Mahe, Seychelles. Phil. Trans. r. Soc., Lond. Ser. B 254: 129-206.
Taylor, J. D., 1970. Intertidal zonation at Aldabra atoll. Phil. Trans. r. Soc., Lond. Ser. B 260: 173-213.
Trevaillon, A., R. R. C. Edwards & J. H. Steel, 1970. Dynamics of a benthic bivalve. In J. H. Steel (ed.), Marine Food Chains. University of Calffornia Press, Los Angeles: 285-295.
Van Someren, V. D. & J. P. Whitehead, 1961. An investigation of the biology and culture of an East African oyster, *Crassostrae cucullata*. Col. Off. Fish. Pub. 14: 1-36.
Vermeij, G. J., 1972. Intraspecific shore-level size gradients in intertidal molluscs. Ecology 53: 693-700.
Vermeij, G. J., 1973. Morphological patterns in high intertidal gastropods: adaptive strategies and their limitations. Mar. Biol. 20: 319-346.
Ward, J. H., 1963. Hierarchical grouping to optimise an objective function. J. am. Stat. Ass. 58: 236-244.
Ward, J. H. & M. E. Hook, 1963. Application of an hierarchical grouping procedure to a problem of grouping profiles. Educational and Psychological Mcasurement 23: 6981.
Younker, J. L. & R. Ehrlich, 1977. Fourier biometrics: harmonic amplitudes as multivariate shape descriptors. Syst. Zool. 26: 336-342.

Ecomorphology of the mangrove oyster *Saccostrea cucullata* (Born, 1778): additional information

Introduction

The mangrove oyster *Saccostrea cucullata* was described for the first time by von Born (1778) as *Ostrea cuccullata.* Because the original spelling *Cuccullata* is orthographically wrong, von Born (1780) corrected the name to *Cucullata* . Following the International Code of Zoological Nomenclature the spelling *cuccullata* can be considered as a *lapsus calami* or unintentional mistake within the meaning of art. 32c ii. This means that *cuccullata* is under consideration for correction to *cucullata* (art. 32d) or emendation (art. 33b ii). Under these circumstances the name of the author and the date of the original spelling are kept. Dollfus & Dautzenberg (1920) placed this species in the genus *Saccostrea.* So the correct name of the mangrove oyster mentioned is *Saccostrea cucullata* (von Born, 1778).
There exists a lot of confusion about the nomenclature of this species. The two main reasons for this problem are:

- the great variation in form;
- the fact that some authors (Thomson, 1954) have regarded *Saccostrea* as a junior synonym of *Crassostrea.* This is believed ot be erroneous, because the two can be distinguished consistently by shell features.

Since 1954 *Crassostrea cucullata* and *Saccostrea cucullata* have been used for the same species. Van Someren & Whitehead (1961) described the biology and the culture of the East African oyster and named it *Crassostrea*

cucullata . Several authors working in the same area adopted this name (Taylor, 1968, 1970; Hartnoll, 1976; Chelazzi & Vannini, 1980; Ruwa, 1984; Okemwa *et al.* , 1986; Vanden Berghe, in press; Tack, 1990). Following the arguments above, preference has to be given to the name *Saccostrea cucullata* (von Born, 1778).
Saccostrea cucullata is found on the trunk and the stilt roots of mangrove trees and rocky substrata in brackish marine environments.
Various studies show intraspecific differences in size of molluscs in relation to environmental parameters (Ebling *et al.* , 1962; Vermeij, 1972, 1973). Okemwa *et al.* (1986) showed that such differences also exist in the mangrove oyster *S. cucullata* growing on a rocky substratum. Previous research also suggested a relationship between the form of the oyster and the substrate. Kesteven (1941) stated that the form of the oyster is partly determined by the form of the substrate. Van Someren & Whitehead (1961) correlated the shape of the oysters with different types of habitats and with different types of substrates. Vanden Berghe (in press) compared specimens of *S. cucullata* on different artificial substrates in a qualitative way. He came to the conclusion that the diameter of the substrate has an influence on the form of the shell.
In this study the effects of different environmental variables on the size and the form of *S. cucullata* growing in a mangrove ecosystem were studied.

Material and methods

Observations were made between July and September 1989 in a small creek near Gazi, a small village 49 km south of Mombasa (Kenya).

Environmental variables

The environmental variables studied were (1) species of the mangrove tree serving as a substrate, (2) diameter of the branch or pneumatophore on which the oyster was growing, (3) approximate density of the oyster, (4) height above the bottom, (5) height above chart datum and (6) orientation with respect to the main tidal current (parallel or perpendicular).
The diameter of the branch serving as a substrate was measured with calipers to the nearest mm. To measure approximate density, we made vertical tran-

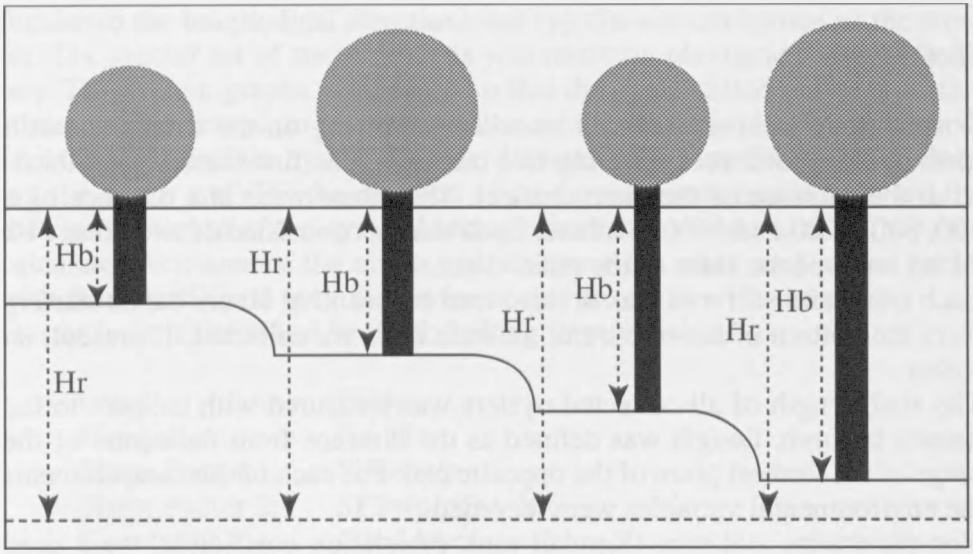

Figure 1. There exists no correlation between height above bottom (Hb) and height above chart datum (Hr).

sects on each of the trunks and/or stilt roots. These transects were made in eight different directions in respect to the main tidal current. The approximate density was expressed as the percentage of the vertical transect that was covered by oysters.

The height above bottom (Hb) was measured to the nearest cm. It is defined as the distance between the middle of the hinge of the oyster and the bottom (base of the tree trunk). Height above chart datum (Hr) was determined following the method of Okemwa *et al.* (1986). In this method, the highest level the water reaches during the tidal cycle is marked. The height of this level is taken to be the same as the one given in the tidal table for the same tidal cycle, and used as an absolute reference for the rest of the measurements. From this reference, height above chart datum was determined to the nearest cm. Figure 1 shows that there is no correlation between height above bottom (Hb) and height above chart datum (Hr). For one mangrove tree of course there is a linear correlation.

Shell length

Observations were made on *S. cucullata* growing on the trunks and stilt roots of mangrove trees covering five transects. The first transect was located at the entrance of the main channel. The others were at a distance of ± 400, 800, 1200 and 1600 m inland. Each transect consisted of two areas (4 x 20 m) on opposite sides of the main channel.
Each trunk and stilt root was divided into consecutive 10 cm bands starting from the bottom of the tree trunk. In each band we collected, if present, an oyster.
The shell length of all collected oysters was measured with calipers to the nearest 0,1 mm. Length was defined as the distance from the centre of the hinge to the farthest point of the opposite end. For each of the measurements the environmental variables were recorded.
Non-parametric statistics (Kendall rank correlation coefficient) were used (Sokal & Rohlf, 1969) to correlate oyster length with the environmental parameters.

Shell form: univalent variables

85 oysters were collected in a restricted area of 10 x 10 m between the second and the third transect. This area was chosen because of the variability of the natural substrates. Only oysters that were growing in isolation (i.e. not touching any other oyster) were included in the sample. In this way, crowding did not interfere with the analysis.
The sample taken was stratified with respect to environmental variables. The environmental variables studied were (1) species of the mangrove tree serving as a substrate, (2) diameter of the branch or pneumatophore on which the oyster was growing, (3) orientation with respect to the main tidal current, (4) height above bottom and (5) height above chart datum. The diameter of the substrate, a continuous variate, was dichotomised by taking oysters growing either on small branches (less than 30 mm diameter) or large branches (more than 100 mm diameter).
To describe the form of the shell we used two sets of measurements. The first set of measurements was made on the shell: (l): oyster length, (w): oyster width (the longest distance between two points on the oyster edge, perpen-

dicular to the longitudinal direction) and (p): the circumference of the oyster. The second set of measurements was made on photographs of the oysters. These photographs were taken so that the oyster filled only 1/9 of the surface of the image, to avoid distortion on the photographs. We measured (A): the surface of the oyster, (Dc): the diameter of the smallest circle enclosing the oyster and (Di) the diameter of the largest circle that is enclosed by the circumference of the oyster. Making use of these measurements, we can calculate (Ac): area of the circle with diameter Dc, (Ai): area of the circle with diameter Di and (pc): circumference of the circle with surface A.
Oyster form is described by the following form variables:

Form:	$F=l/w$
Elongation:	$E=w/l$
Shape Factor 1:	$SF1=pc/p$
Shape Factor 2:	$SF2=(p/pc) \times 100$
Circularity 1:	$C1=4A/p^2$
Circularity 2:	$C2=4A/lp$
Compactness 1:	$c1=2(pA)^{1/2}$
Compactness 2:	$c2=p^2/4pA$
Circularity 3:	$C3=(A/Ac)^{1/2}$
Shape Factor 3:	$SF3=Ai/A$
Shape Factor 4:	$SF4=(Ac-Ai)/A$
Shape Factor 5:	$SF5=(A/Ac) \times 100$
Form Relation:	$FR=A/l^2$
Ellipticity Index:	$EI=(0,5pl^2)A$

Non-parametric statistics (Kendall rank correlation coefficient) were used to correlate these univalent form variables to the environmental factors measured.

Shell Form: Fourier analysis

The photographs of the 85 oysters were used to describe the circumference of the oyster shell by means of Fourier analysis. The photographs of the oysters were magnified and the circumference of the shell was redrawn on paper. These drawings were digitized using a Complot series 7000 digitizer, coupled to an IBM AT. The resolution of the digitizer was 0,025 mm and

points were taken at intervals of 0,8 mm around the perimeter. The average length of the digitized circumference was about 1 m.

Fourier analysis is a technique used for the quantitative description of continuous periodical functions. Closed contours can be transformed into continuous periodical functions. This means that Fourier analysis can be used to describe closed contours in a quantitative way.

A closed contour can be quantified using Fourier analysis, by considering the polar coordinates of the points of the contour, with the origin of the polar coordinates chosen within the contour (e.g. Ehrlich & Weinberg, 1970; Kaesler & Waters, 1972; Anstey & Delmet, 1972, 1973; Christopher & Waters, 1974; Younker & Ehrlich, 1977; Healy-Williams & Williams, 1981). This method was also used to describe the form of oysters by Vanden Berghe (in press).

A limitation of the method involving polar coordinates is that only those forms where there is only one intersection between the boundary and a ray from the origin of the polar coordinates can be analysed. The form of most of the oysters is quite irregular and does not satisfy this condition. Hence another method, the elliptical method, has been used in this study. For this method, both x and y coordinates of the boundary are interpreted as a function of distance along the boundary. The two resulting functions are periodical, with a period given by the length of the boundary, and can be separately analysed using the Fourier transform (Kuhl & Giardini, 1982; Ferson *et al.* , 1985).

The analysis was performed on an IBM AT. Before performing the transformation, a total of 64 equally spaced points were interpolated between the digitized points. For each of the forms to be analysed, 32 harmonics (128 Fourier coefficients) were calculated. Preliminary tests showed that, with this number of harmonics, all the features of the form of the circumference could be described accurately.

The coefficients resulting from the Fourier transform were entered in a cluster analysis to detect groups of oysters of similar form.Ward's method (Ward, 1963; Ward & Hook, 1963) was used for clustering. In this method, the distance measure is the squared Euclidean distance. The method of aggregation is a modification of the weighted average method; the dispersion of the elements of a cluster around its centre of gravity is taken into account when determining which clusters are to be combined.

The cluster analysis was performed with an IBM AT, using the program

SPSS/PC. The distances between different groups are given in arbitrary units. Since only relative distances are important here, this does not interfere with the interpretation of the results.

Results

Shell length

The species of mangrove tree, the diameter of the branch serving as a substrate and the orientation with respect to the main tidal current appeared to be of no importance for shell length.

The Kendall rank correlation coefficient shows that the rank correlation between the oyster length and height above chart datum is very low (τ=-0.092; n=2826; $p \leq 10^{-5}$). However, Figure 2 shows a clear relation between the height above chart datum and the oysters with maximum length. Oysters

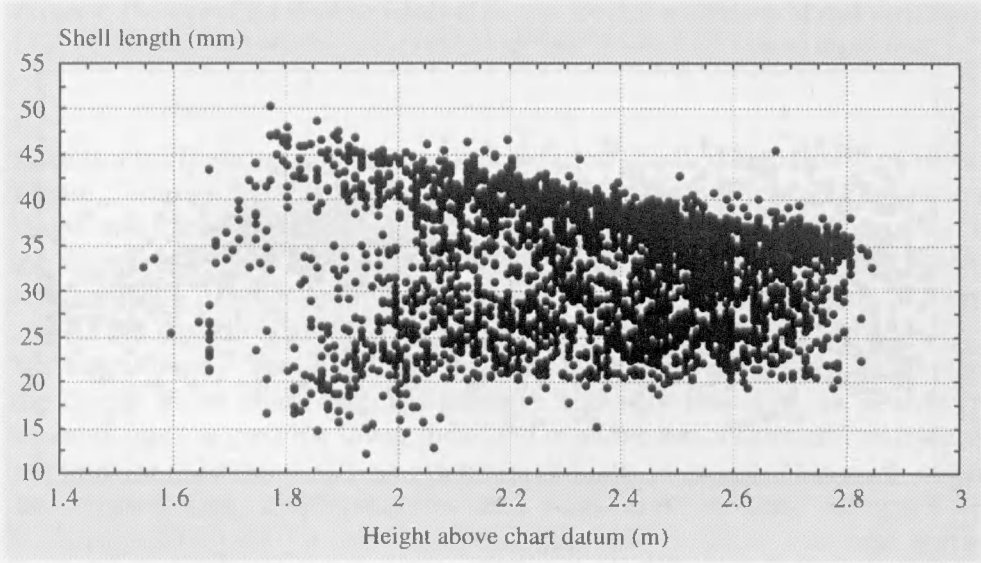

Figure 2. Saccostrea cucullata. Relation between the oyster length (mm) and the height above datum (m) (τ=-0.092; n=2862; $p \leq 10^{-5}$).

that do not reach the maximum length grow where there is a high density of oysters and/or close to the base of the tree trunk.
Figure 3 shows the weak rank correlation between the length and the approximate density of the oysters (τ=-0.207; n=2862; $p \leq 10^{-5}$). This figure shows a clear difference between the lower and higher approximate densities. For the lower approximate densities there is a positive relationship between oyster length and the approximate densities. The opposite is true for the higher approximate densities. To decide where the dataset has to be split we split it at every possible percentage of approximate density of the oysters, starting at 1% and increasing it by 1% every time. For each percentage we calculated the Kendall coefficients of rank correlation for the two parts (Kl and Kr) of the split dataset. When the sum of the absolute values of the two Kendall coefficients of rank correlation became maximal, we found the most sensible percentage to split the dataset (see Fig. 4). The rank correlation between oyster length and the approximate density of the oysters is weak up to a density of 65% (τ=0.162; n=1837; $p \leq 10^{-5}$). For the densities higher than 65%

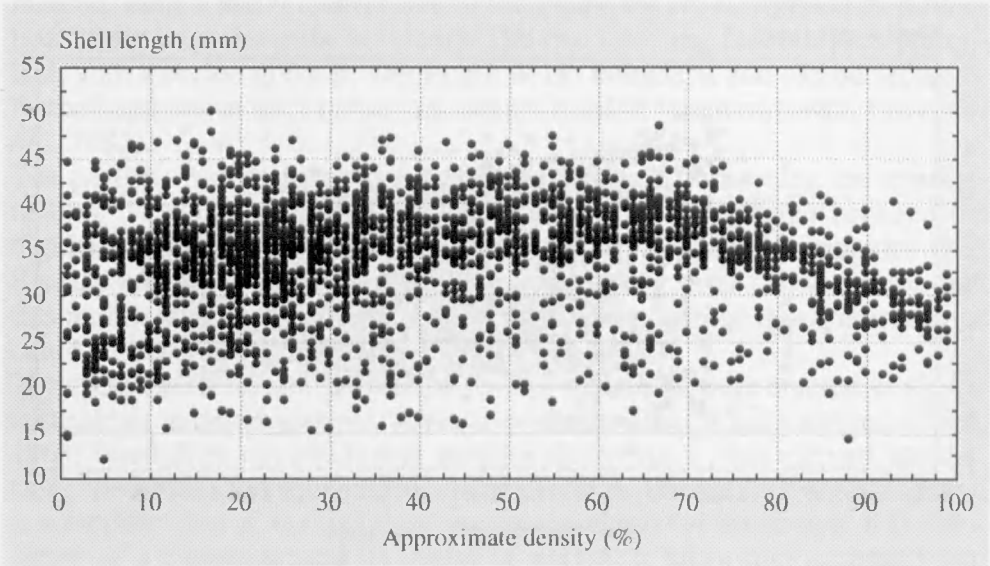

Figure 3. Saccostrea cucullata. Relation between the oyster length (mm) and the approximate density (%) of the oysters (τ =-0.207; n=2862; $p \leq 10^{-5}$).

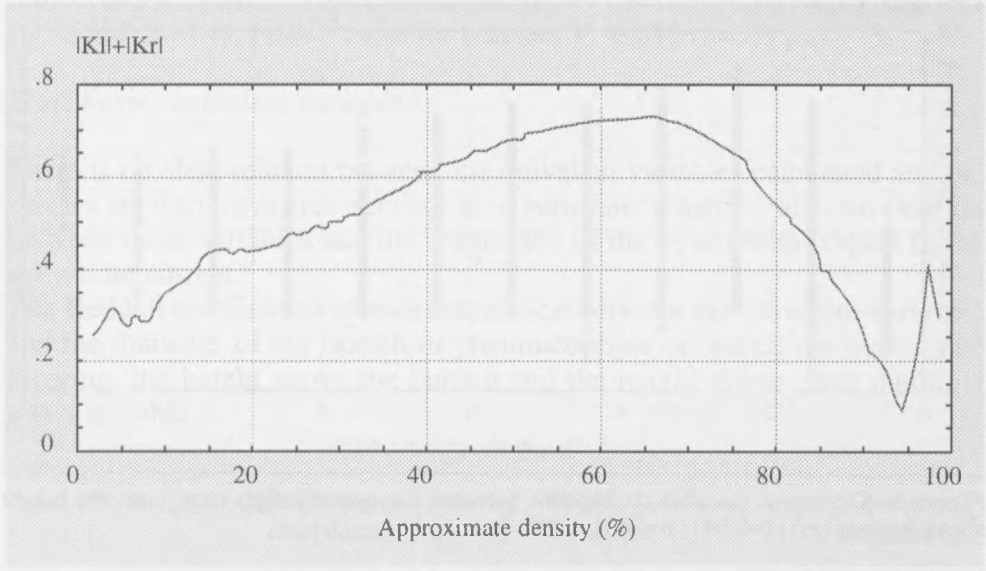

Figure 4. The sum of the absolute values of the two Kendall coefficients of rank correlation (|K|| + |Kr|). The most sensible percentage to split the dataset is 65%.

there is a fairly strong negative rank correlation (τ=-0.556; n=1049; $p \leq 10^{-5}$). Figure 5 shows an analogous situation in the relation between the oyster length and the height above bottom. The optimal height above datum to split the dataset is 0.25 m. The rank correlation between oyster length and height above bottom is 0.471 (n=952; $p \leq 10^{-5}$) for height above the bottom ≤0.25 m and -0.148 (n=2384; $p \leq 10^{-5}$) for heights above the bottom ≥0.25 m.

We have already mentioned that the correlation between oyster length and the height above chart datum is negative and very low (Fig. 2). However, when all oysters growing lower than 0.25 m above the bottom and all oysters growing on substrates with an approximate density greater than 65% cover are removed from the dataset, the rank correlation becomes stronger (τ=-0.522; n=1401; $p \leq 10^{-5}$) (see Fig. 6). The Kendall coefficient of rank correlation becomes even stronger when the dataset is divided into 5, each of them containing the measurements of one of the transects. The Kendall coefficient of rank correlation for transect I is -0.785 (n=492; $p \leq 10^{-5}$), for transect II -

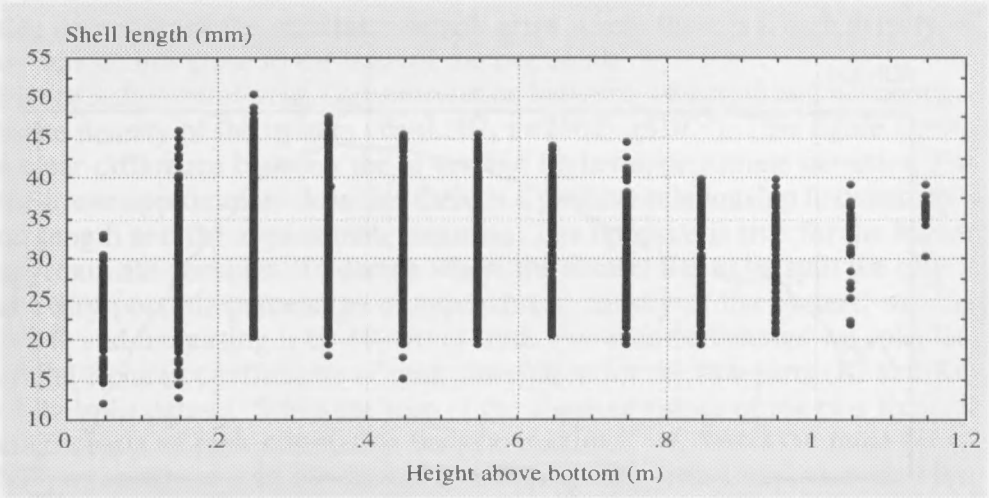

Figure 5. Saccostrea cucullata. Relation between the oyster length (mm) and the height above bottom (m) (τ=0.041; n=2862; $p \leq 10^{-3}$).

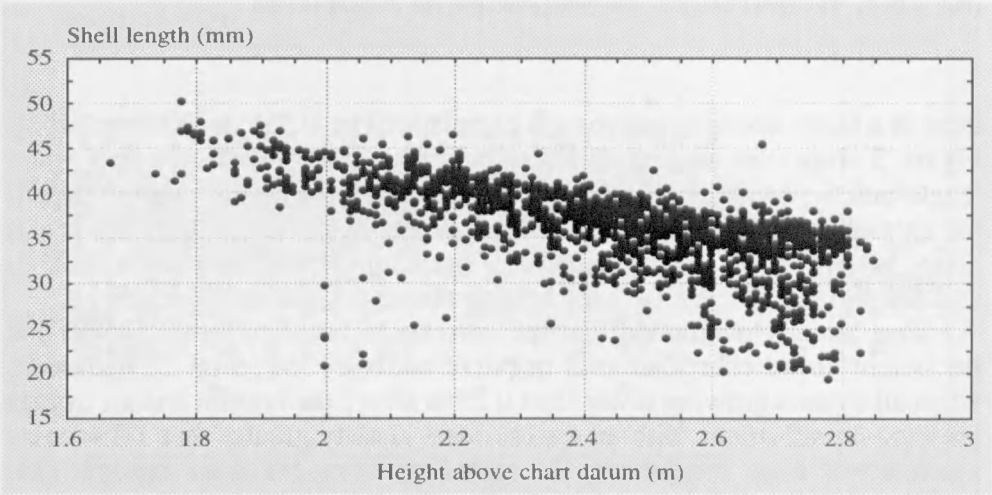

Figure 6. Saccostrea cucullata. Relation between the oyster length (mm) and the height above chart datum (m). Oysters growing lower than 0.25 m above the bottom and all oysters growing on substrates with an approximate density greater than 65% cover are removed of the dataset. (τ=-0.522: n=1401; $p \leq 10^{-5}$)

0.627 (n=530; $p \leq 10^{-5}$), for transect III -0.749 (n=182; $p \leq 10^{-5}$), for transect IV -0.637 (n=144; $p \leq 10^{-5}$) and for transect V -0.636 (n=53; $p \leq 10^{-5}$).

Shell Form: univalent variables

There is no clear relation between the univalent variables calculated and the species of mangrove tree serving as a substrate. There is also no relation between these variables and the orientation of the oyster with respect to the main tidal current.
The Kendall coefficients of rank correlation between the univalent variables and the diameter of the branch or pneumatophore on which the oyster was growing, the height above the bottom and the height above chart datum is given in Table 1.

	Height above chart datum	Height above bottom	Diameter substratum
F	- 0.047	0.036	- 0.113
E	0.047	- 0.036	0.113
SF1	- 0.726*	- 0.242*	0.032
SF2	0.726*	0.242*	- 0.032
C1	- 0.726*	- 0.242*	0.032
C2	- 0.601*	- 0.231*	0.106
c1	- 0.726*	- 0.242*	0.032
c2	0.726*	0.242*	-0.032
C3	0.056	- 0.050	0.138
SF3	0.065	0.032	0.056
SF4	- 0.072	0.019	- 0.121
SF5	0.056	- 0.050	0.138
FR	0.026	- 0.058	0.127
EI	- 0.026	0.058	- 0.127

Table 1. Saccostrea cucullata. Rank correlation between univalent variables of shell form and environmental variables (*: $p \leq 0,01$)

Shell Form: Fourier analysis

The ecological and morphological variables of the 85 oysters used in this study are given in Table 2a and 2b. The coefficients resulting from the Fourier transform were entered in a cluster analysis. The cluster dendrogram resulting from the cluster analysis is shown in Figure 7. This cluster dendogram divides the 85 oysters into different groups of oysters of similar form. A first division of the sample into groups of more or less equal size occurred at the distance level 25. These two groups coincided with oysters that occurred high above chart datum or low above chart datum. Oysters growing in intermediate levels (between 3.05 and 3.13 m above chart datum) occurred in both groups of cluster analysis.
Each of the two above mentioned groups was separated into two subgroups, on distance levels of 9 and 14 respectively. These two divisions coincided with the oysters growing on small or on large diameters of substrate. Only one misclassification occurred for this criterion. The interpretation for the three other environmental variables (species of mangrove tree serving as a substrate, orientation with respect to the main tidal current and height above bottom) was not as straightforward, in the sense that there was no strict dichotomy subdividing each of the groups on levels lower than the one discussed above. However, it was still possible to assign a group characteristic to each of the groups of the cluster analysis with respect to orientation, if only two categories are considered (parallel or perpendicular). If the group characteristic is taken to be the value of the majority in the group, 13 misclassifications occurred.

Discussion

Shell length

The oyster length was weakly correlated (rank correlation) with the approximate density of the oysters. However, for densities equal to and higher than 65% there was a fairly strong negative correlation. This demonstrates the influence of density on oyster length for densities higher than 70% cover. The influence increases as density increases. Other authors have demon-

Oyster n°	Substratum (species of mangrove tree)	Orientation with respect to the main tidal current	Diameter substratum (mm)	Oyster length (mm)	Oyster width (mm)	Height above the bottom (m)	Height above chart datum (m)
1	A	a	124	36.80	17.20	0.72	2.66
2	A	a	11	41.95	15.85	0.51	2.42
3	A	b	14	42.55	28.30	0.69	3.14
4	A	a	167	37.50	19.75	0.69	2.92
5	A	z	236	29.80	24.70	1.21	3.18
6	A	b	151	34.90	24.25	0.29	3.35
7	A	b	190	28.50	15.85	0.37	2.80
8	S	a	137	33.35	20.70	0.86	3.15
9	S	b	210	28.75	19.75	1.08	3.42
10	S	a	18	25.95	21.10	0.44	2.40
11	S	a	178	36.45	19.15	0.99	3.11
12	S	b	21	32.60	23.05	0.18	3.07
13	R	z	14	42.50	15.30	0.33	2.95
14	R	a	7	39.95	22.65	0.49	2.42
15	R	a	139	37.15	19.75	0.31	3.26
16	R	a	18	43.30	37.45	0.79	3.20
17	R	z	27	40.85	34.40	0.36	2.45
18	R	a	19	28.50	31.65	0.16	3.11
19	R	b	406	32.15	24.60	1.34	3.39
20	R	a	24	35.60	36.65	0.81	2.95
21	R	b	143	34.85	31.75	0.41	2.71
22	R	z	24	38.20	28.85	0.54	3.22
23	A	z	19	41.95	28.70	1.18	3.18
24	B	a	13	25.75	13.85	0.90	3.26
25	B	a	25	36.35	26.90	0.63	3.16
26	B	b	24	30.35	18.05	0.43	3.07
27	B	b	23	30.95	18.80	1.04	3.13
28	B	a	17	43.70	22.75	0.54	2.81
29	B	z	30	30.45	24.80	0.58	2.26
30	B	b	28	38.65	24.60	0.62	2.24
31	B	b	114	40.25	31.10	0.32	2.67
32	B	b	140	25.05	26.00	0.41	2.97
33	B	b	101	26.10	20.40	1.13	3.29
34	B	b	20	35.15	23.15	0.61	2.89
35	B	a	18	39.75	27.25	0.50	2.41
36	B	b	30	24.20	20.55	0.09	3.19
37	B	b	17	30.05	12.55	1.28	3.37
38	B	z	11	23.25	11.00	0.42	2.86
39	S	z	30	35.00	24.85	0.94	3.34
40	S	b	19	39.10	30.60	0.33	3.42
41	S	z	23	27.35	20.70	1.13	3.17
42	S	z	184	36.40	24.20	0.49	2.60
43	S	z	19	34.25	17.80	0.35	2.94

Table 2a. Saccostrea cucullata : environmental and morphological variables of the oysters studied (A: *Avicennia marina* ; B: *Bruguiera gymnorrhiza* ; R: *Rhizophora mucronata* ; S: *Sonneratia alba* ; a & b: oysters growing perpendicular to the main tidal current; z: oysters growing parallel to the main tidal current)

Oyster n°	Substratum (species of mangrove tree)	Orientation with respect to the main tidal current	Diameter substratum (mm)	Oyster length (mm)	Oyster width (mm)	Height above the bottom (m)	Height above chart datum (m)
44	A	b	151	34.00	26.35	0.29	3.35
45	A	a	130	42.10	21.35	1.13	3.18
46	A	z	124	35.75	19.95	0.72	2.66
47	A	z	29	38.95	38.60	0.83	2.91
48	A	b	21	32.35	25.25	1.13	3.35
49	A	z	10	30.30	23.95	0.18	3.07
50	R	z	19	39.15	31.15	0.98	3.34
51	R	b	14	32.55	27.10	0.84	2.93
52	R	z	22	40.95	22.75	0.55	2.77
53	R	z	9	33.80	29.30	0.53	2.39
54	R	z	18	35.25	21.30	0.17	3.19
55	R	a	9	34.70	25.05	0.89	3.18
56	R	z	25	40.25	28.80	0.28	3.35
57	R	a	236	36.35	34.10	0.37	2.72
58	R	a	9	35.95	27.75	0.51	3.17
59	R	a	26	32.10	28.50	0.88	3.31
60	R	b	21	34.60	35.05	0.49	3.13
61	R	b	12	32.85	26.70	0.54	3.20
62	R	b	127	32.15	25.10	0.31	2.78
63	B	a	28	30.30	22.80	0.23	3.14
64	B	a	7	34.15	12.10	0.74	3.24
65	B	b	26	22.75	17.55	1.07	3.14
66	B	z	14	24.50	12.35	0.93	3.32
67	B	b	29	26.10	18.35	0.56	2.41
68	B	z	18	26.65	19.65	0.23	3.02
69	B	a	18	37.40	26.70	1.04	3.06
70	A	z	187	37.45	32.20	1.26	3.32
71	A	z	134	40.94	44.30	0.98	3.06
72	A	z	24	32.70	31.80	0.24	3.14
73	A	b	18	39.40	35.15	1.38	3.33
74	A	a	236	34.35	31.35	0.20	3.12
75	A	a	167	25.55	21.50	1.05	3.17
76	A	a	128	28.90	29.35	1.00	3.37
77	S	a	26	37.05	24.75	0.48	2.74
78	S	a	26	48.65	29.10	0.77	3.12
79	S	a	6	36.70	36.20	1.11	3.32
80	S	b	16	34.30	26.15	1.00	3.41
81	S	b	17	46.80	23.30	0.92	3.01
82	S	z	107	38.35	29.25	0.45	3.22
83	S	z	106	30.30	27.10	0.69	2.79
84	S	z	22	32.25	29.85	0.42	3.03
85	S	z	26	31.30	25.80	0.17	2.20

Table 2b. Saccostrea cucullata : environmental and morphological variables of the oysters studied (A: *Avicennia marina* ; B: *Bruguiera gymnorrhiza* ; R: *Rhizophora mucronata* ; S: *Sonneratia alba* ; a & b: oysters growing perpendicular to the main tidal current; z: oysters growing parallel to the main tidal current)

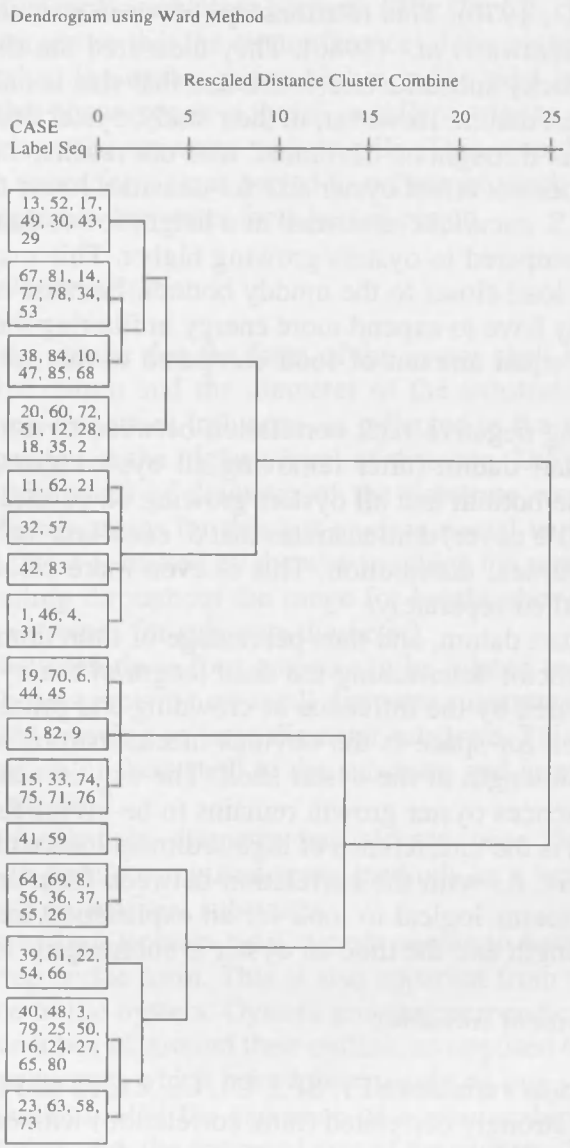

Figure 7. Saccostrea cucullata. Shell form, Fourier analysis: cluster dendrogram.

strated that density may affect growth rate and size in some littoral bivalves (Trevallion *et al.* , 1970). This relationship (between size and density) was not found by Okemwa *et al.* (1986). They measured the shell length of *S. cucullata* on a rocky substrate and concluded that size is only a function of height above chart datum. However, in their study, oyster density was smaller than 70% cover throughout. Combined with our results, this indicates that oyster density does not affect oyster size for densities lower than 70% cover. Shell lengths of *S. cucullata* are small at a height lower than 0.25 m above the bottom as compared to oysters growing higher. This may be due to the higher sediment load closer to the muddy bottom. Because oysters are filter feeders, they may have to expend more energy in filtering the water at lower levels to get an equal amount of food compared to the oysters growing at higher levels.

The fairly strong negative rank correlation between oyster length and the height above chart datum (after removing all oysters growing lower than 0.25 m above the bottom and all oysters growing on substrata with a density higher than 70% cover) demonstrates that *S. cucullata* exhibits size-related patterns in vertical distribution. This is even more clear when the five transects are studied separately.

Height above chart datum, and thus percentage of time immersed, seems to be the primary factor determining the shell length of the oysters. This relationship is obscured by the influence of crowding and proximity to the bottom. Competition for space is the obvious mechanism by which crowding can influence the length of the oyster shell. The way in which proximity to the bottom influences oyster growth remains to be investigated, but a probable mechanism is the interference of high sediment load with the filter feeding of the oysters. As with the correlation between form and height above chart datum, it seems logical to look for an explanation for the correlation between shell length and the time an oyster is submerged (feeding time).

Shell form: univalent variables

The univalent form variables SF1, SF2, C1, C2, c1, c2 and the height above chart datum are strongly correlated (rank correlation) with each other. Those formulas have one thing in common: p, the circumference of the oyster. SF1, C1, C2 and c1 decrease when the circumference of the oyster increases. This means that the shell form becomes more irregular. SF2 and c2 decrease when

the shell form becomes more irregular.
The strong negative rank correlation between SF1, C1, C2, c1 and the height above chart datum shows that the circumference of the oyster shell becomes more irregular when the oyster grows higher in the tidal level. A possible explanation for this phenomenon is that *S. cucullata* tries to enlarge the contact surface between the seawater and its gills. This would enable oysters which are in the water for a short period to collect as much food as oysters which are submerged in the water for a longer period.

Shell form: Fourier analysis

It is clear from the results that the form of the oyster shell is influenced by both height above datum and the diameter of the substrate. Height above datum has the most important influence, as reflected in the fact that the two groups were separated at the highest level of distance. Differences of form resulting from differences of diameter of the substrate were smaller. The number of misclassifications for this last environmental variable was small in this study, but this was caused by the way in which the sampling was done (continuous sampling throughout the range for height above datum, versus sampling on the extremes for substrate diameter).
The distinction between these four groups can be related to the appearance of the oysters. Oysters growing on small diameter substrates are more elongated than the ones growing on large diameter substrate. This allows them to attach a large portion of their shell to the substrate and improves the structural strength of the shell.
The influence of substrate diameter has already been demonstrated by Vanden Berghe (in press), using analogous methods on a very small sample of oysters growing on artificial substrates.
Orientation with respect to main tidal current seems to have a weaker, but still clear influence on the form. This is also apparent from a visual inspection of the outline of the oysters. Oysters growing perpendicular to the tidal current often have lobes all around their outline, as opposed to oysters growing parallel to the current, which have lobes mainly on one side.
This study has demonstrated the existence of a relationship between environmental parameters and, the form and size of the oysters. The next step in research should be the construction of a functional model to investigate the causal relationship between shell morphology of *S. cucullata* and the environmental variables.

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References

ANSTEY R. L., DELMET D. A., *Genetic Meaning of Zooecial Chamber Shapes,* in: "Science", 177, 1972, p. 1000-1002.

ANSTEY R. L., DELMET D. A., *Fourier Analysis of Zooecial Shapes in Fossil Tubular Bryozoans,* in: "Geol. Soc. Am. Bull.", 84, 1973, p. 1753-1764.

CHELAZZI G., VANNINI M., *Zonation of Intertidal Molluscs on Rocky Shores of Southern Somali,* in: "Estuar. coast. mar. Sci.", 10, 1980, p. 569-583.

CHRISTOPHER R. A., WATERS J. A., *Fourier Series as a Quantitative Descriptor of Miospore Shape,* in: "J. Paleont.", 48, 1974, p. 697-709.

DOLLFUS G.-F., DAUTZENBERG P., *Conchyliologie du Miocène moyen du bassin de la Loire, pt. 1: Pélécypodes (Suite et fin),* in: "Paleontologie", 22, 1920, p. 379-500.

EBLING F. J., SLOANE J. F., DAVIES H. M., *The Ecology of Lough Ine. XII. The Distribution and Characteristics of Patella Species,* in: "J. anim. Ecol.", 31, 1962, p. 457-470.

EHRLICH R., WEINBERG B., *An Exact Method for Characterization of Grain Shape,* in: "J. Sed. Petrol.", 40, 1970, p. 205-212.

FERSON S., ROHLF F. J., KOEHN R. K., *Measuring Shape Variation of Two-Dimensional Outlines,* in: "Syst. Zool.", 34, 1985, p. 59-68.

HARTNOLL R. G., *The Ecology of Some Rocky Shore in Tropical East Africa,* in: "Estuar. coast. mar. Sci.", 4, 1976, p. 1-21.

HEALY-WILLIAMS N., WILLIAMS D.F., *Fourier Analysis of Test Shape of Planktonic Foraminifera,* in: "Nature", 289, 1981, p. 485-487.

KAESLER R. L., WATERS J. A., *Fourier Analysis of the Ostracode Margin,* in: "Geol. Soc. Am. Bul.", 83, 1972, p. 1169-1178.

KESTEVEN G. L., *The Biology and Cultivation of Oysters in Australia. I. Some Economic Aspects,* Commonwealth of Australia, Melbourne, 1941.

KUHL F. P., GIARDINA C. R., *Elliptic Fourier Features of a Closed Contour,* in: "Computer Graphics and Image Processing", 18, 1982, p. 236-258.

OKEMWA E., RUWA R. K., POLK P., *The Autecology of the Edible Oyster Crassostrea cucullata Born, 1778: Size Related Vertical Distribution at Mkomani, Mombasa,* in: "Kenya J. Sci. Ser. B", 7, 1986, p. 9-14.

RUWA R. K., *Invertebrate Faunal Zonation on Rocky Shores around Mombasa, Kenya,* in: "Kenya J. Sci. Ser. B", 5, 1984, p. 49-65.

SOKAL R. R., ROHLF F. J., *Biometry. The Principles and Practise of Statistics in Biological Research,* W. H. Freeman & Co, San Francisco, 1969.

TACK J. F., *De ecomorfologie van de mangroveoester Crassostrea cucullata,* Licentiaatsverhandeling Vrije Universiteit Brussel, 1990.

TAYLOR J. D., *Coral Reef Associated Invertebrate Communities (Mainly Molluscan) around Mahe, Seychelles,* in: "Phil. Trans. r. Soc., Lond. Ser. B", 254, 1968, p. 129-206.

TAYLOR J. D., *Intertidal Zonation at Aldabra Atoll,* in: "Phil. Trans. r. Soc., Lond. Ser. B", 260, 1970, p. 173-213.

THOMSON J. M., *The Genera of Oysters and the Australian Species,* in: "Australian Jour. Mar. & Freshwater Res.", 5, 1954, p. 132-168.

TREVALLION A., EDWARDS R. R. C., STEEL J. H., *Dynamics of a Benthic Bivalve,* in: "Marine Food Chains", Steel J. H. (ed.), 1970, p. 285-295.

VANDEN BERGHE E., *The Form of Oysters in Relation to the Form of the Substrate,* in: "Symposium Proceedings of the HYSEA Annual Symposium on State of Knowledge and Recent Research Advances in Freshwater and Marine Biology in Eastern Africa", in press.

VAN SOMEREN V. D., WHITEHEAD J. P., *An Investigation of the Biology and Culture of an East African Oyster, Crassostrea cucullata,* in: "Col. Off. Fish. Pub.", 14, 1961, p. 1-36.

VERMEIJ G.J., *Intraspecific Shore-Level Size Gradients in Intertidal Molluscs,* in: "Ecology", 53, 1972, p. 693-700.

VERMEIJ G.J., *Morphological Patterns in High Intertidal Gastropods: Adaptive Strategies and their Limitations,* in: "Mar. Biol.", 20, 1973, p. 319-346.

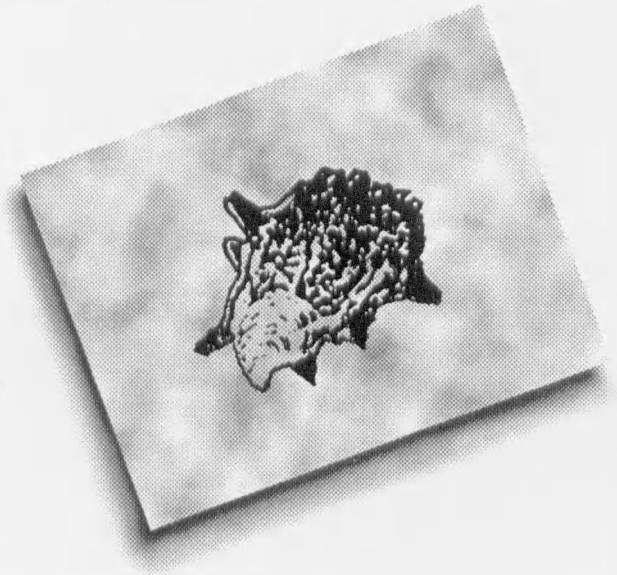
VON BORN I., *Index rerum naturalium musei Caesarei Vindobonensis, Pars I'.ma, Testacea,* in: "Verzeichniss der natürlichen Seltenheiten des K. K. Naturalien Kabinets zu Wien, Erster Theil, Shaltiere", Officina Krausiana, Vienna, 1778.

VON BORN I., *Testacea musei Caesarei vindobonensis quae jussu Mariae Theresiae Augustae disposuit et descripsit,* Joannus Paulus Kraus, Vienna, 1780.

WARD J. H., *Hierarchical Grouping to Optimize an Objective Function,* in: "J. am. Stat. Ass.", 58, 1963, p. 236-244.

WARD J. H., HOOK M. E., *Application of an Hierarchical Grouping Procedure to a Problem of Grouping Profiles,* in: "Educational and Psychological Measurements", 23, 1963, p. 69-81.

YOUNKER J. L., EHRLICH R., *Fourier Biometrics: Harmonic Amplitudes as Multivariate Shape Descriptors,* in: "Syst. Zool.", 26, 1977, p. 336-342.



Chapter

3

THE MANGROVE ECOSYSTEM

Groundwater flow in the coastal zone influences mangrove distribution

Abstract

Mangroves are a major protection in the tropics against coastal erosion, are nursery grounds for a variety of marine animals and increase sedimentation of particles brought to the coast by rivers. Mangrove distribution in the tropics and subtropics is often linked with the presence of estuaries and creeks. There is a consensus that the brackish water micro environment is caused by river discharges into the oceans. However all over the world mangrove areas are found where no rivers or estuaries are in the immediate neighbourhood. An explanation is reported here for a number of those exceptions. Using a mathematical groundwater flow model it is shown how human activities as far as several hundreds of kilometres inland can destroy vast areas of mangroves by changing the groundwater flow. The model predicts and/or confirms the destruction of large mangrove areas in Kenya and in Florida (USA).

Introduction

Walsh (1974) suggested the existence of extensive mangal depended upon five basic factors. Chapman (1975, 1977, 1984) believed there are seven: (1) air temperature, (2) ocean currents, (3) protection from wave action, (4) shallow shores, (5) salt water, (6) tidal range, and (7) substrate.

Mangrove distribution in the tropics and subtropics is often linked with the presence of estuaries and creeks (Macnae, 1968; Barth, 1982; Blasco, 1991). There is a consensus that the brackish water micro environment, which is the key factor for the development of mangroves, is caused by river discharges into the oceans (Macnae, 1968; Barth, 1982; Snedaker, 1982).

However all over the world mangrove areas are found where no rivers or estuaries are in the immediate neighbourhood. In this study an explanation is presented for a number of those exceptions making use of a mathematical groundwater flow model. Figure 1 shows the distribution of mangroves along the Kenyan coast. Most of the mangrove areas are in the proximity of one or more rivers. However, the rivers north of Mombasa are perennial while the rivers south of Mombasa are drying up after the rainy season. On the other hand there are a number of mangrove areas with no rivers in the immediate neighbourhood. The mangrove forest of Mida Creek (between Kilifi and Malindi, Kenya) is a clear example of a mangrove forest growing in an area without a visible freshwater source.
For regions whose groundwater flow pattern is not known, the use of mathematical models can be a powerful tool in predicting unknown variables, e.g. the impact of a changing groundwater flow pattern on the mangrove ecosystem. The model used is able to explain mangrove distribution in two different areas (Kenyan coast and the Everglades National Park in Florida (USA)). Also the usefulness of the model in predicting mangrove destruction in the above mentioned mangrove areas is shown.
This study describes the groundwater flow of two different regions by a mathematical groundwater model (Ituli, 1984; Dapaah-Siakwan, 1986). Such a model consists of a set of mathematical differential equations with their boundary and initial conditions, and a numerical solution procedure. Groundwater flow takes place according the gradient of piezometric heads. This means that by solving the model equation for piezometric heads and by knowing their distribution in the study areas the groundwater flow pattern can be established.

Material and methods

Groundwater model

The mathematical model used in this study was developed by the Laboratory of Hydrology of Brussels Free University (De Smedt and Bronders, 1985). The model used is designed to simulate the response of a phreatic, semi-confined or confined aquifer to an imposed stress. The model allows homogeneous or heterogeneous aquifers with irregular boundaries. The model also

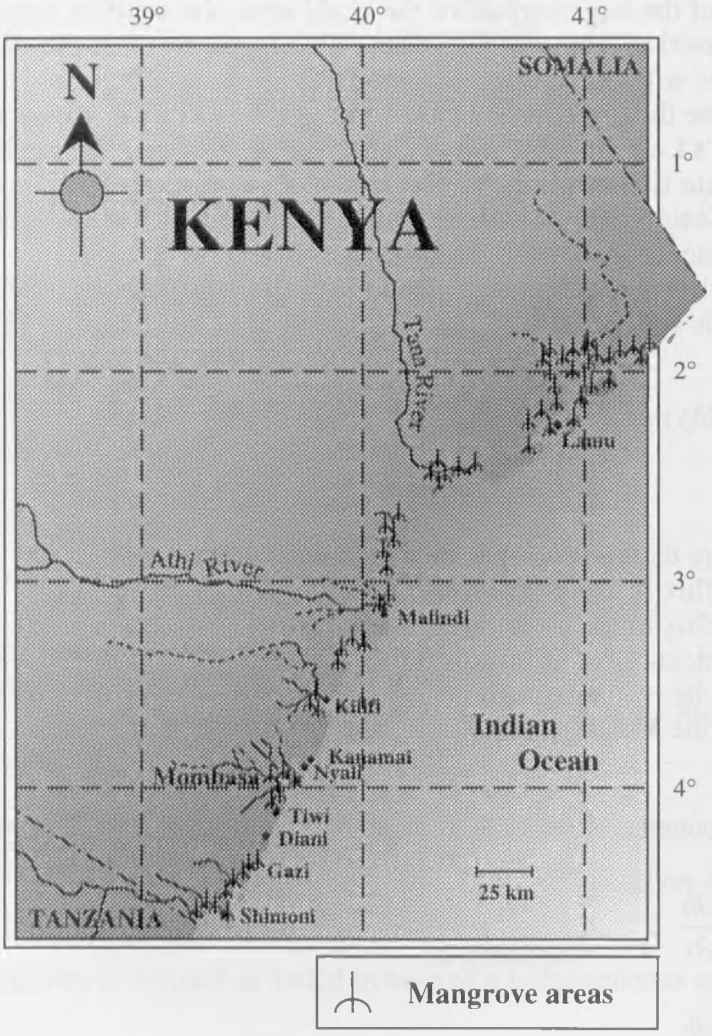


Figure 1. Distribution of mangrove forests along the Kenyan coast.

allows constant/variable recharge, constant/variable and surface inflow or outflow. The groundwater flow is considered horizontal in the model. Because of the large surface of the study areas the aquifers vary in geological composition from place to place. This implicates that the transmissivity also varies with the geologic composition of the aquifer.
To describe the groundwater flow through porous media, the model is based on Darcy's Law and the Law of Conservation of Mass. The model considers steady-state two-dimensional, horizontal flow through a non-homogeneous isotropic aquifer of variable thickness, including source and sink terms, as groundwater recharge and withdrawal.
To formulate the governing equation for groundwater flow, the Continuity equation is applied to an elemental volume within the aquifer (Fig. 2):

$$\frac{\partial q_x}{\partial x}\Delta x(d\Delta y)+\frac{\partial q_y}{\partial y}\Delta y(d\Delta x)=R(x,y)\Delta x\Delta y-Q(x,y)\Delta x\Delta y \qquad \text{(equation 1)}$$

where
x and y are the horizontal Cartesian co-ordinates;
q_X is the flux in the x-direction;
q_Y is the flux in the y-direction;
d is the thickness of the aquifer;
R(x,y) is the recharge; and
Q(x,y) is the surface outflow.

The components of the groundwater flux are obtained by Darcy's law:

$$q_x=-K\frac{\partial h}{\partial x} \qquad \text{(equation 2)}$$

$$q_y=-K\frac{\partial h}{\partial y} \qquad \text{(equation 3)}$$

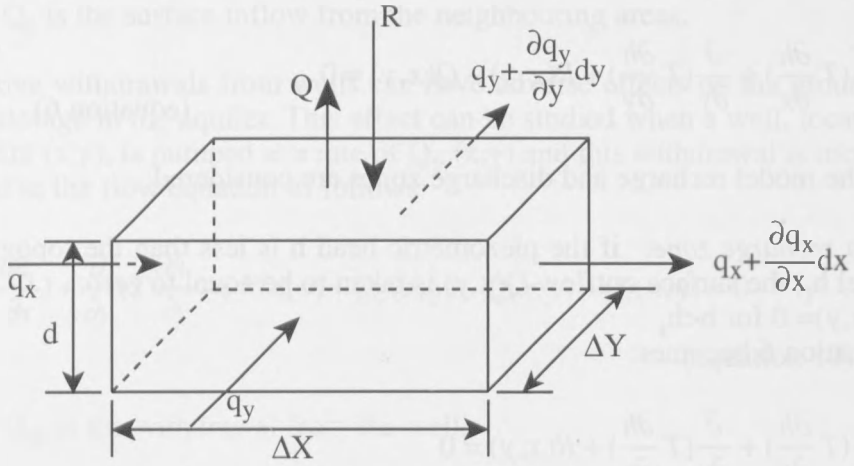


Figure 2. An elemental volume within the aquifer.

where
K is the hydraulic conductivity; and
h is the piezometric head or groundwater elevation.
Equation 1, after substitution of equitions 2 and 3 and after dividing by $\Delta x \Delta y$, becomes:

$$\frac{\partial}{\partial x}(-Kd\frac{\partial h}{\partial x})+\frac{\partial}{\partial y}(-Kd\frac{\partial h}{\partial y})=R(x,y)-Q(x,y) \qquad \text{(equation 4)}$$

The transmissivity is defined as T=Kd in case of a homogeneous aquifer, or as

$$T=\int_0^d Kdz \qquad \text{(equation 5)}$$

in case of a layered medium, such that equation 4 can be written as:

$$\frac{\partial}{\partial x}(T\frac{\partial h}{\partial x})+\frac{\partial}{\partial y}(T\frac{\partial h}{\partial y})+R(x,y)-Q(x,y)=0 \qquad \text{(equation 6)}$$

In the model recharge and discharge zones are considered:

A. a recharge zone: if the piezometric head h is less than the topographic level h_t, the surface outflow Q(x,y) is taken to be equal to zero:
$Q(x,y)= 0$ for $h<h_t$
Equation 6 becomes:

$$\frac{\partial}{\partial x}(T\frac{\partial h}{\partial x})+\frac{\partial}{\partial y}(T\frac{\partial h}{\partial y})+R(x,y)=0 \qquad \text{(equation 7)}$$

B. a discharge zone: if the piezometric head h is equal to or tends to be greater than the topographic level h_t, the surface outflow Q(x,y) is taken to be greater than zero, so that the piezometric head becomes equal to the topographic level:
$Q(x,y)>0$ for $h=h_t$
Equation 6 becomes:

$$\frac{\partial}{\partial x}(T\frac{\partial h_t}{\partial x})+\frac{\partial}{\partial y}(T\frac{\partial h_t}{\partial y})+R(x,y)=Q(x,y) \qquad \text{(equation 8)}$$

If surface outflow occurs, the model considers the water to flow downwards to the adjoining areas with lower topographic levels. This water is than added to the normal recharge input in these areas.
The flow equation now becomes:

$$\frac{\partial}{\partial x}(T\frac{\partial h_t}{\partial x})+\frac{\partial}{\partial y}(T\frac{\partial h_t}{\partial y})+R(x,y)-Q(x,y)+Q_s(x,y)=0 \qquad \text{(equation 9)}$$

where Q_S is the surface inflow from the neighbouring areas.

Excessive withdrawals from wells can have adverse effects on the groundwater storage in the aquifer. This effect can be studied when a well, located at a point (x,y), is pumped at a rate of $Q_W(x,y)$ and this withdrawal is incorporated in the flow equation as follows:

$$\frac{\partial}{\partial x}(T\frac{\partial h_t}{\partial x})+\frac{\partial}{\partial y}(T\frac{\partial h_t}{\partial y})+R(x,y)-Q_w(x,y)-Q(x,y)+Q_s(x,y)=0$$

(equation 10)

where Q_W is the withdrawal from the well.

In order to solve the groundwater flow equation, we need to specify the boundary and the initial conditions. A groundwater flow domain can be defined by several types of boundary conditions. In the regions studied, use is made of two boundary conditions:

Potential boundary conditions
In this type of boundary conditions the piezometric heads are known: h=h*(x,y) where h* is a known piezometric head for all points along the boundary. In the model this type of boundary represents that part of the aquifer where the piezometric head would not change in time. In natural conditions, such boundary conditions occur as recharge boundaries or areas beyond the influence of hydraulic stresses and are defined by known equipotential lines.

No flow boundary conditions
These boundaries are defined by a line across which no flow is occurring, thus

$$\frac{\partial h}{\partial x}=0$$

(equation 11) or

$$\frac{\partial h}{\partial y} = 0 \qquad \text{(equation 12)}$$

This means that perpendicular to the boundary no flow is occurring. This kind of boundary can be defined in nature by two situations: the existence of an outcropping of impervious rock, or a groundwater divide.

Initial conditions
The groundwater flow equation describes a steady state situation so no initial condition is required. However, the topographic levels are required to calculate the piezometric heads in the study area, and to identify the discharge and recharge zones.

The central finite difference approximation method is used to solve the partial differential equation describing the groundwater flow. The model equations describing the regional groundwater flow are solved by a computer programme originally written in FORTRAN IV (Ituli, 1984; Dapaah-Siakwan, 1986). For this application it has been rewritten in C++, and also a number of graphic outputs were added.

Input
The basin characteristics that serve as inputs for the computer to solve the model equations are the transmissivity values T, the areal net precipitation R, the topographic levels h_t, the aquifer thickness d and the porosity of the aquifer material n.
The transmissivity values are obtained by the product of hydraulic conductivity and the thickness of the aquifer: T=Kd, where K is the hydraulic conductivity and d is the thickness of the aquifer. The flow domains are divided into several zones having different transmissivity values. Those zones correspond to the geological units distinguished in the flow domains. Transmissivity data of Kenya were obtained from Ituli (1984) and are shown in Figure 3. Transmissivity data of Florida were obtained from the Water Resources Research Center (Florida). In this study the transmissivity data for Florida were kept constant at a mean value of 0.242 10^4 m^2/day for the whole study area.

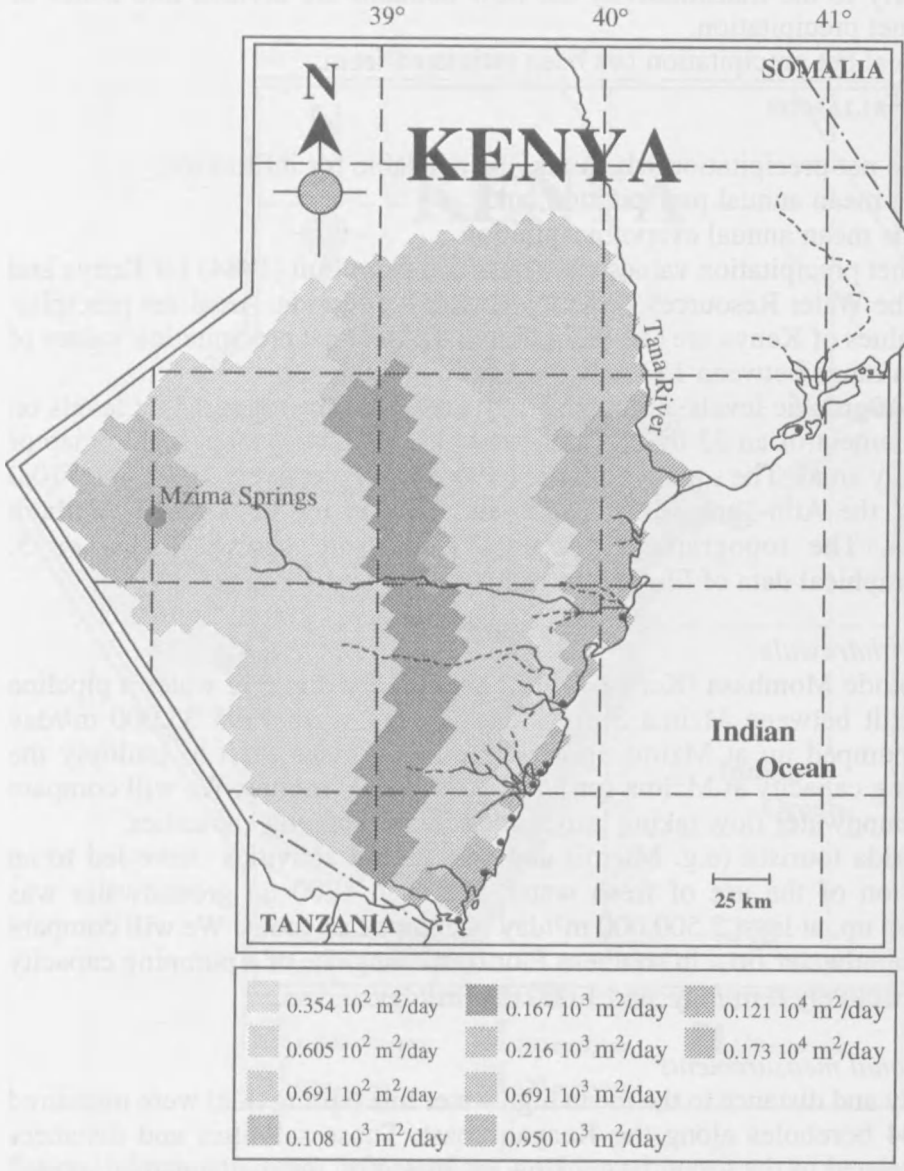


Figure 3. Zones with equal mean transmissivity (m^2/day) in the study area (Kenya).

Similarly to the transmissivity the flow domains are divided into zones of equal net precipitation.
The areal net precipitation has been estimated from:
$R=P - E_t$
where
R is the net precipitation which may be available for infiltration;
P is the mean annual precipitation; and
E_t is the mean annual evapotranspiration.
Areal net precipitation values were obtained from Ituli (1984) for Kenya and from the Water Resources Research Center for Florida. Areal net precipitation values of Kenya are shown in Figure 4. Areal net precipitation values of Florida range between 130 mm/year and 690 mm/year.
The topographic levels are obtained by averaging the topographic levels on each element of an 33 by 38 grid system imposed on a topographic map of the study areas. The square grids are measuring respectively 10.0 km by 10.0 km for the Athi-Tana River Basin, and 7.9 km by 7.9 km for Southern Florida. The topographical data of Kenya are shown in Figure 5. Topographical data of Florida range between 0 and 45 m.

Well withdrawals
To provide Mombasa (Kenya) with the necessary drinking water a pipeline was built between Mzima Springs and Mombasa. In 1995 35.000 m^3/day were pumped up at Mzima springs. Presently, plans exist to multiply the pumping capacity at Mzima springs up to 350.000 m^3/day. We will compare the groundwater flow taking into account both pumping capacities.
In Florida tourism (e.g. Miami) and agricultural activities have led to an explosion of the use of fresh water. While in 1900 no groundwater was pumped up, at least 2.500.000 m^3/day is pumped up today. We will compare the groundwater flow in southern Florida making use of a pumping capacity of respectively 0 m^3/day and 1.000.000 m^3/day.

Additional measurements
Salinity and distance to the mean high water line (spring tide) were measured for 104 boreholes along the Kenyan coast. Those salinities and distances were related to the groundwater flow estimated by the mathematical model. Individual relations between groundwater flow and respectively salinity and distance to the mean high water line (spring tide) were studied making use

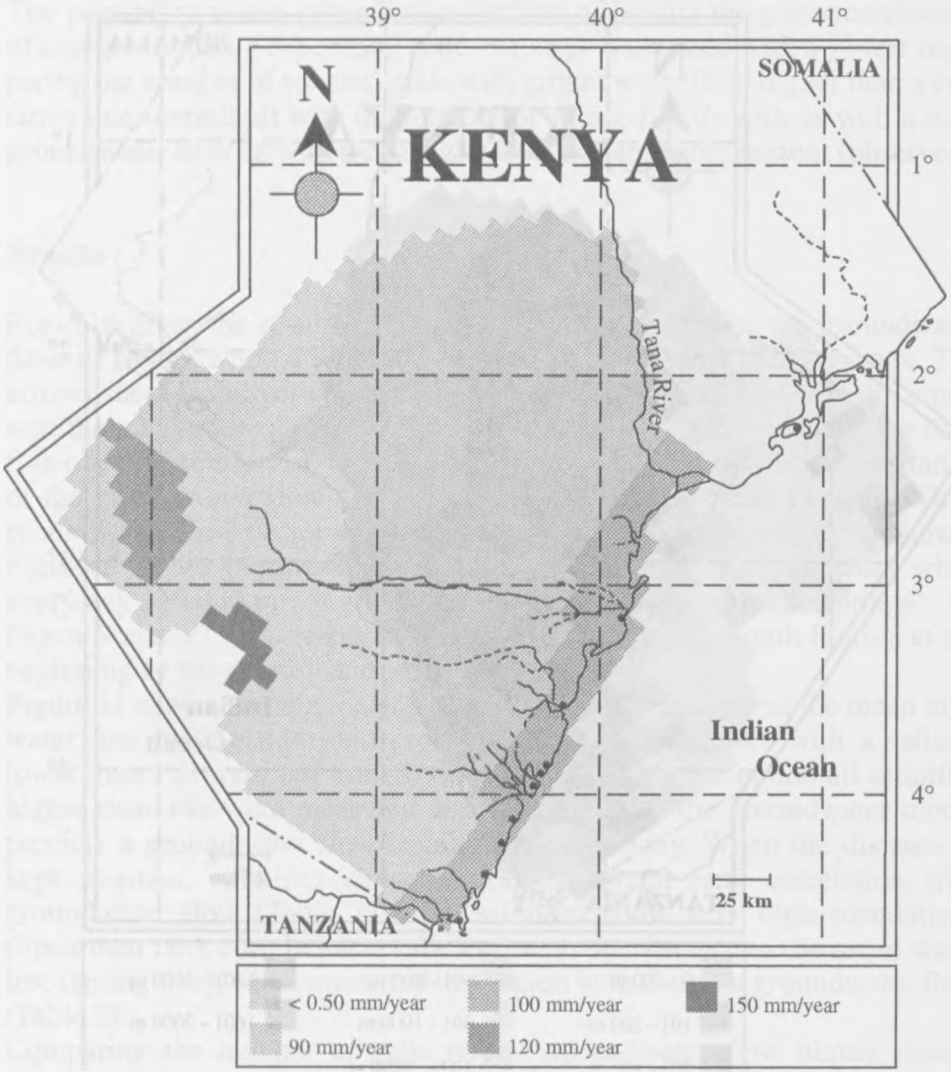


Figure 4. Zones with equal mean areal net precipitation (mm/year) in the study area (Kenya).

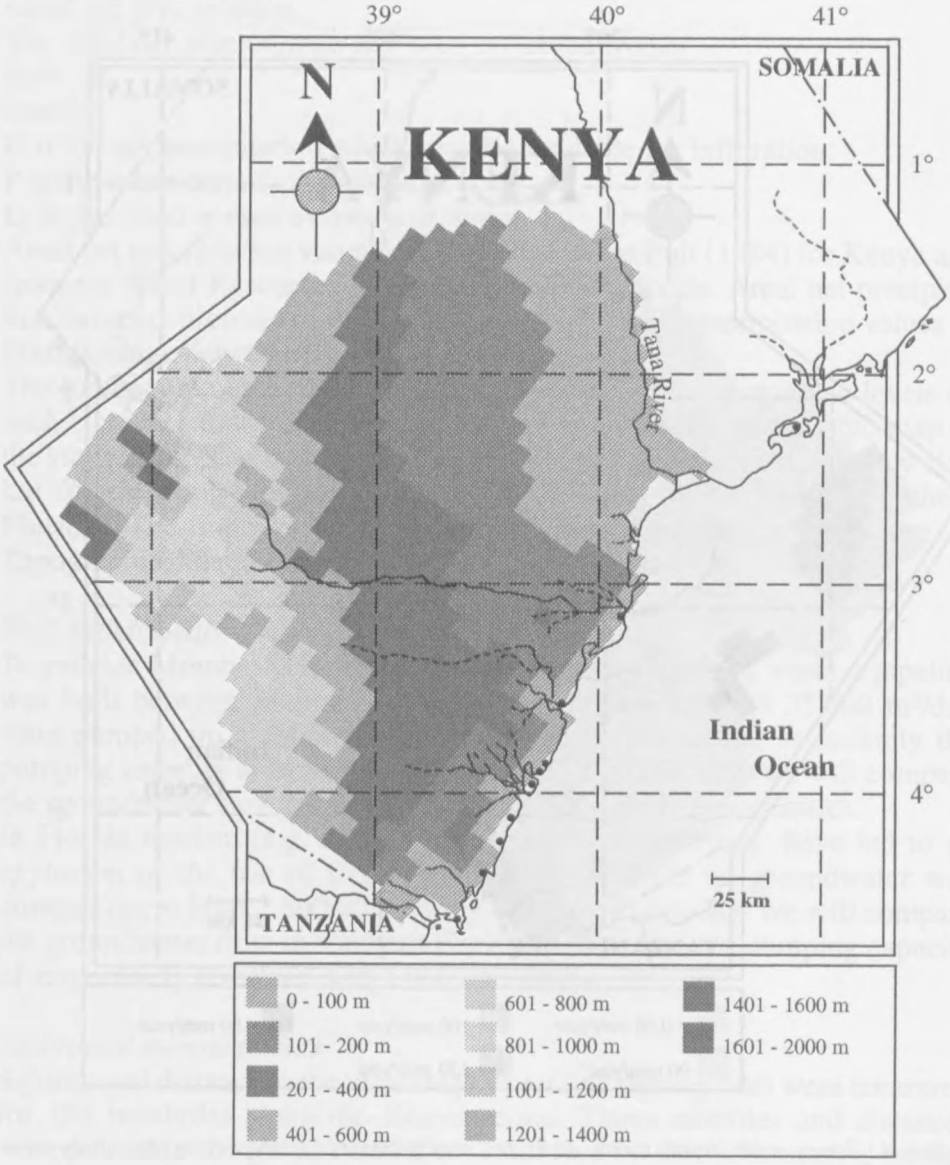


Figure 5. Zones with equal mean topographic levels (m) in the study area (Kenya).

of a Spearman rank correlation coefficient.
The possibility to use groundwater outflow to predict the presence/absence of mangroves along the coastal zone of Kenya was tested with a χ^2-test comparing the number of squared grids with groundwater flow higher than a certain value (expected) with the number of squared grids with as well a high groundwater flow as with the mangrove ecosystem being present (observed).

Results

Figure 6 gives the results of the mathematical model for the groundwater flow in Kenya when 35.000 m^3/day is pumped up at Mzima springs. The arrows show the direction of the groundwater flow, while the colours represent the magnitude of the groundwater flow, which is expressed as the total flux over the aquifer, i.e. flux times aquifer tickness. A vector representation of the groundwater flow along the coastal zone is given in Figure 7. Only groundwater flow vectors with a groundwater flow ≥ 1 m^2/day are shown. Figure 8 shows groundwater flow vectors along the Kenyan coast when every day 350.000 m^3 of groundwater is pumped up at Mzima springs.
Figure 9 and 10 show respectively groundwater flow in South Florida at the beginning of the century and in 1994.
Figure 11 shows the relationship between salinity, distance to the mean high water line (spring tide) and groundwater flow. Boreholes with a salinity lower than 1‰ were not used in the graph. With one exception all salinities higher than 1‰ were measured at locations where the groundwater model predicts a groundwater flow smaller than 1 m^2/day. When the distance is kept constant, salinities show a clear Spearman rank correlation with groundwater flow (Table 1). Also salinities show very high correlations (Spearman rank correlation coefficient) with the distance to the mean water line (spring tide), when measured in a region with constant groundwater flow (Table 2).
Comparing the number of cells with a groundwater flow higher than 1 m^2/day with the number of cells where the groundwater flow is higher than 1 m^2/day and where the mangrove forest is present, a χ^2 test shows no significant differences.

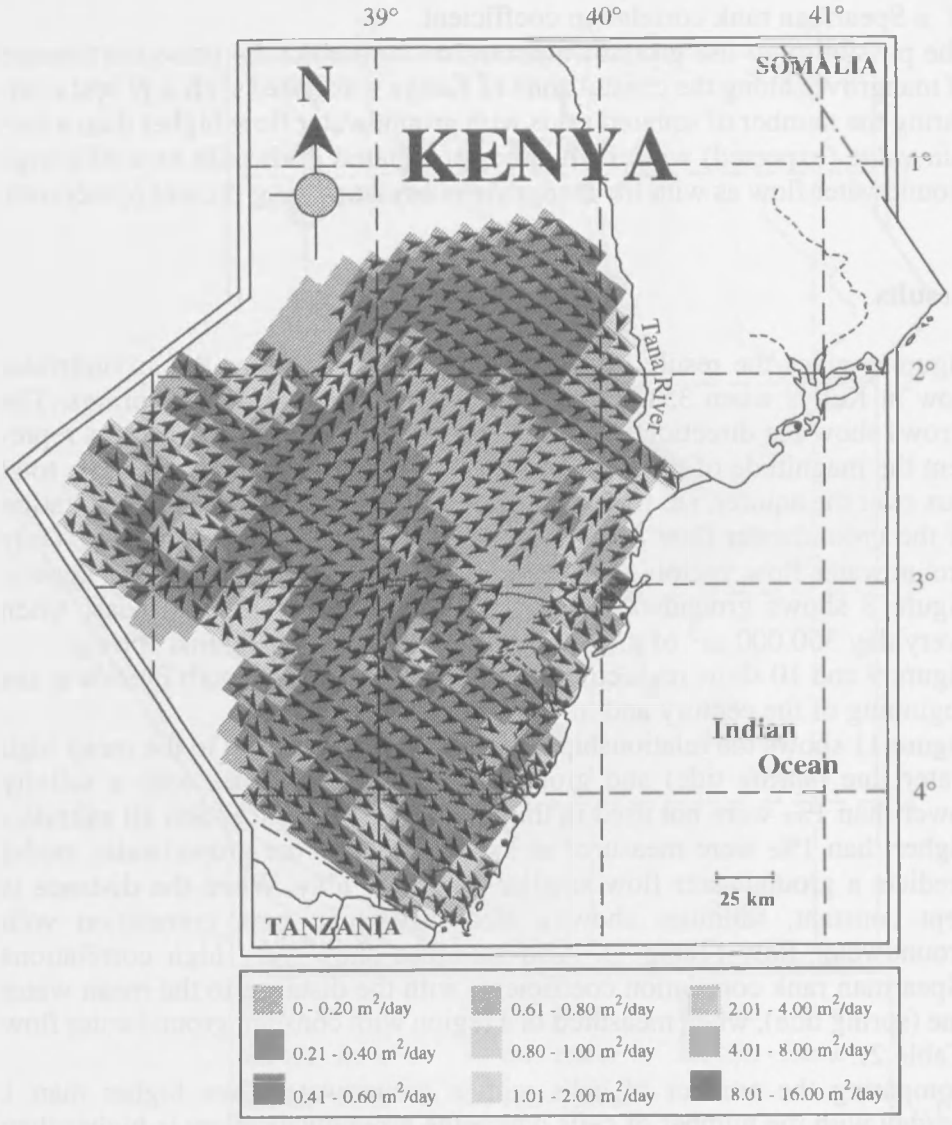


Figure 6. Groundwater flow: graphical output of the model. Arrowheads show the direction of the groundwater flow. Background colours indicate the size of the groundwater flow (m^2/day).

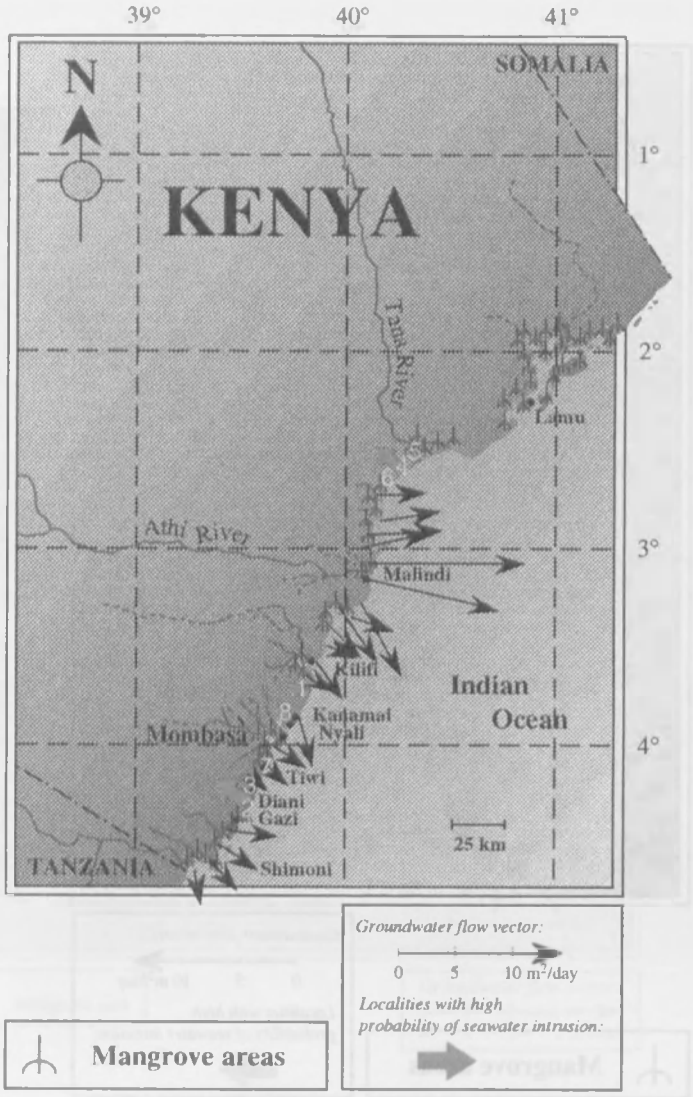


Figure 7. Groundwater flow: graphical output (vectorial) of the actual situation along the Kenyan coast between the Tanzanian border and Tana River. Only those areas with groundwater flow higher than 1 m^2/day and regions with a high probability of seawater intrusion are indicated. Numbers 1 to 8 refer to salinity measurements at different distances from the coast (see Fig. 11).

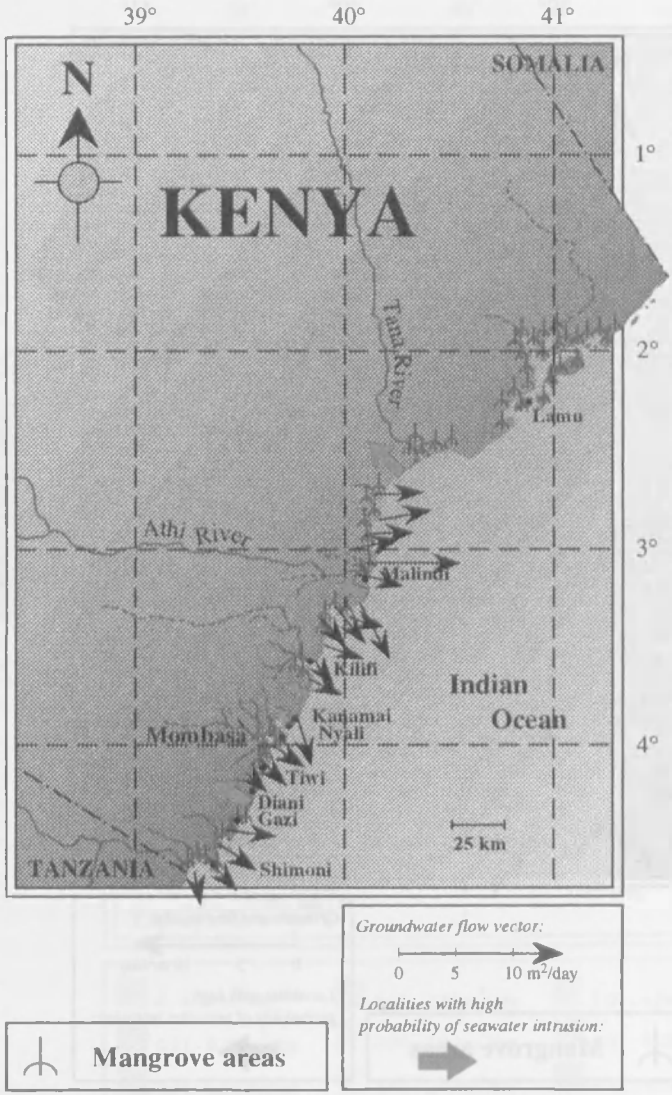


Figure 8. Groundwater flow: graphical output (vectorial) of the modelled situation along the Kenyan coast between the Tanzanian border and Tana River when 350.000 m^3/day is pumped at Mzima springs. Only those areas with groundwater flow higher than 1 m^2/day and regions with a high probability of seawater intrusion are indicated.

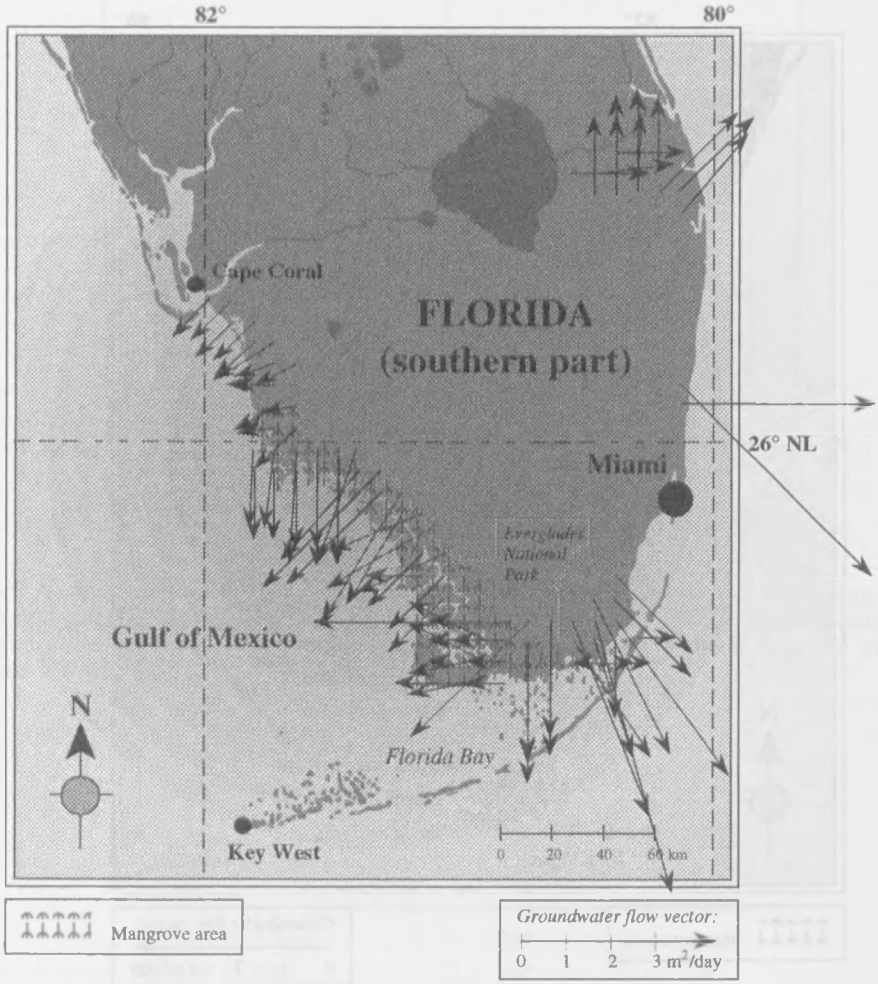


Figure 9. Groundwater flow: graphical output (vectorial) of the groundwater flow situation in the southern part of Florida in the beginning of the 19th century. In the western part of the study region groundwater flow is high in the direction of the Gulf of Mexico. In the eastern part there are only a few areas with high groundwater flow.

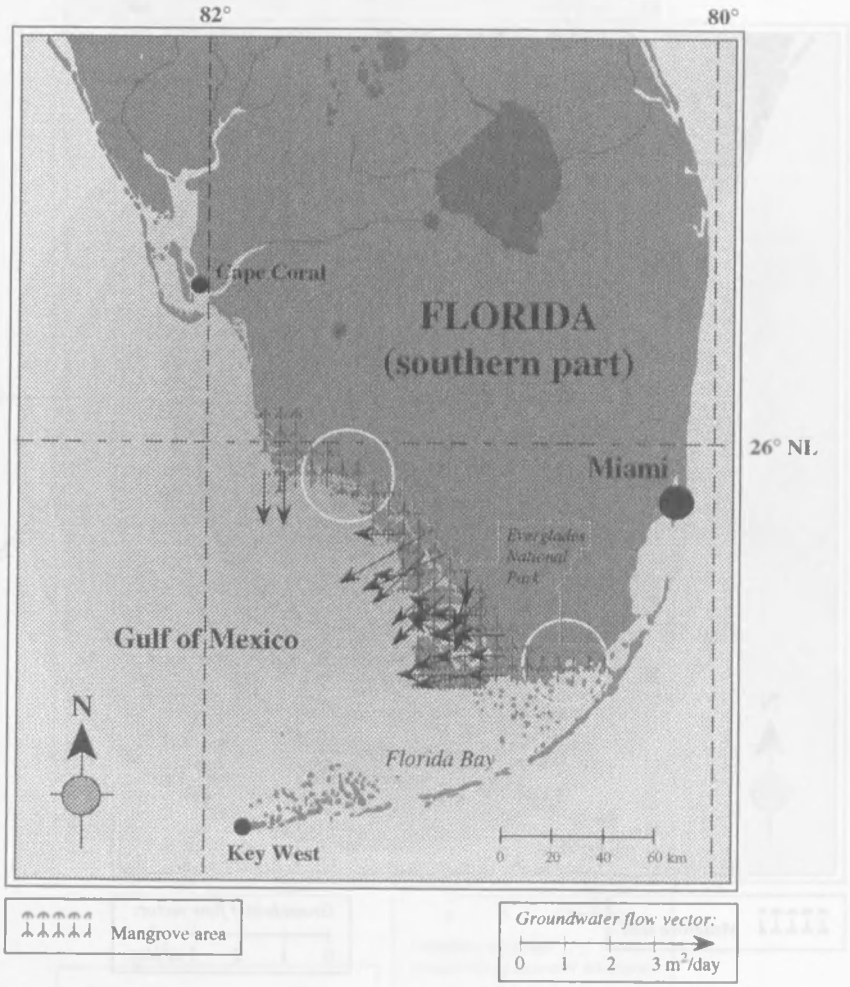


Figure 10. Groundwater flow: graphical output (vectorial) of the groundwater flow in the southern part of Florida based on actual data (Feb. 1994).

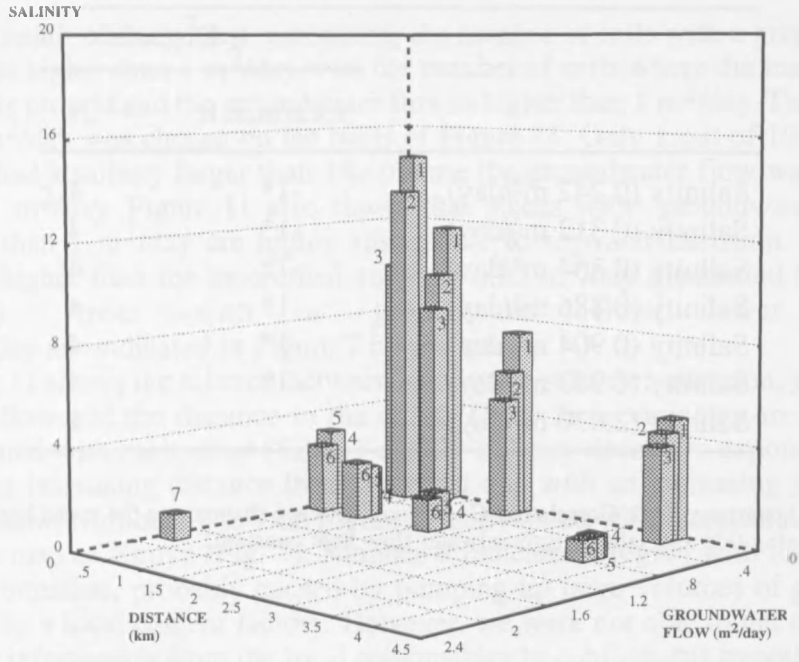


Figure 11. Relation between salinity (‰), distance to the mean high water line (spring tide) (km) and groundwater flow (m^2/day). Measurements taken in 104 boreholes along the Kenyan coast. Boreholes with a salinity lower than 1‰ were not added to the graph. Numbers 1 to 8 refer to places along the Kenyan coast as given in Figure 7.

	Groundwater flow	
	Spearman R	N
Salinity (0.5 km)	-.78*	26
Salinity (1 km)	-.73*	26
Salinity (2 km)	-.74*	26
Salinity (4 km)	-.74*	26

Table 1. Spearman Rank Correlation Coefficient between groundwater flow and salinity (distance to the mean high water line at spring tide kept constant).

	Distance	
	Spearman R	N
Salinity (0.242 m^2/day)	-1*	4
Salinity (0.312 m^2/day)	-1*	4
Salinity (0.354 m^2/day)	-1*	4
Salinity (0.886 m^2/day)	-1*	4
Salinity (0.904 m^2/day)	-1*	4
Salinity (0.963 m^2/day)	-1*	4
Salinity (2.030 m^2/day)		1

Table 2. Spearman Rank Correlation Coefficient between distance to the mean high water line at spring tide and salinity (groundwater flow kept constant).

Discussion and conclusions

The coastal zone of Kenya is characterised by moderately high groundwater flow ranging between 0.31 and 12.8 m^2/day (average value: 4 m^2/day). The high groundwater flow is due to high potential gradients, high infiltration capacity of the geologic formations and high precipitation received by the area.

The elevation of the coastal belt ranges between 0 and 76 m. Piezometric heads as calculated from the model range between 0 and 48 m. In general those piezometric heads reflect the field situation: wells in the coastal belt strike water between 5 and 15 m.

High discharge of fresh groundwater into the sea is in conformity with Cashwell and Bakers 's (1953) assertion that close to the shores, in some points, seepage of freshwater occurs. Seawater seepage is reported in several areas along the Kenyan coast (Isaac and Isaac, 1968; Knutzen and Jasuund (1979); Ruwa and Polk, 1986; Ruwa, 1993).

Figure 7 shows a very clear correlation between groundwater flow and the distribution of the mangroves along the coast. This correlation is confirmed

by the result of the χ^2 test comparing the number of cells with a groundwater flow higher than 1 m^2/day with the number of cells where the mangrove forest is present and the groundwater flow is higher than 1 m^2/day. The value of 1 m^2/day was chosen on the basis of Figure 11. Only 1 out of 104 boreholes had a salinity larger than 1‰ in case the groundwater flow was more than 1 m^2/day. Figure 11 also shows that places with groundwater flow lower than 1 m^2/day are highly susceptible to seawater intrusion. This is much higher than the theoretical value of 0.22 m^2/day mentioned by Ituli (1984). Areas with a groundwater flow lower than 1 m^2/day are indicated in Figure 7 by red arrows.

Figure 11 shows the relation between salinity, or seawater intrusion, groundwater flow and the distance to the coast. Those three variables are clearly correlated with each other (Table 1 and 2). Salinity decreases exponentially with an increasing distance from the coast and with an increasing groundwater flow. Numbers 1 to 7 on Figure 11 correspond with identical numbers on the map of Kenya (Fig. 7). Number 8 indicates a region with local seawater intrusion, probably caused by pumping up large volumes of groundwater by a local cement factory. However, we were not able to get the necessary information from the local responsibles to confirm this hypothesis.

To solve the drinking-water problem of Mombasa, plans exist to pump up more water at Mzima Springs. Pumping of huge amounts of water will lead to an alteration of the regional groundwater flow along the coast. At the moment groundwater is pumped at a rate of 35.000 m^3/day. If this amount would be raised to the planned 350.000 m^3/day the groundwater flow in the region of Malindi and in the region south of Tana River would decrease drastically (Fig. 8). As a consequence, the probability of seawater intrusion would increase in both regions, and the existing mangrove forests would be endangered, because seawater intrusion would lead to the destruction of the ecosystem.

To prove the capability of the model to predict the destruction of the mangrove forest due to lower groundwater flows, we tested the model for the southern part of Florida, with a special interest to the Everglades National Park. The groundwater flow in the beginning of the century was compared to the situation in 1994. Figure 9 (beginning of this century) shows large groundwater outflow in the southern and western part of the region. In Figure 10 (situation 1994) the groundwater flow in the whole area has dropped drastically, because of urbanisation, tourism and agriculture. In

those places where groundwater flow fell below 1 m^2/day, mangroves are dying, as indicated by the yellow circles in Figure 10.
The complete absence of groundwater flow in the south-eastern part of the park together with the diminished surface water flow (Holloway, 1994; Fennema *et al.,* 1994), also explanains the doubling of the salinity in Florida Bay and the sea grass die-off in this bay (Mairson, 1994).
Hence, this study shows a clear relation between the distribution of the mangrove ecosystem and groundwater flow. Groundwater modellisation seems to be a proper tool to evaluate the effects of massive pumping of groundwater. The model also shows how human activities as far as several hundreds of kilometres inland can destroy vast areas of mangroves by changing the groundwater flow. The model predicts and/or confirms the destruction of large mangrove areas in Kenya and the Everglades (Florida-USA). The present authors believe at least one variable must be added to the list of seven basic conditons (Chapman, 1975, 1977, 1984) for the existance of extensive mangrove forests: presence of fresh water.

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References

Barth, H. (1982). The biogeography of mangroves. In: Sen, D. N., Rasjpurohit, K. S. (Ed.) Tasks for Vegetation Science 2. Dr. W. Junk Publishers, The Hague, p. 35-59
Blasco, F. (1991). Les mangroves. La Recherche 231: 444-453
Cashwell, P. V., Baker, B. H. (1953). Geology of the Mombasa-Kwale Area. Bulletin Geol. Surv. Kenya, Report No. 24, Nairobi
Chapman, V. J. (1975). Mangrove biogeography. In: Walsh, G. D., Snedaker, S. C., Teas, H. J. (Ed.) Proceedings International Symposium on the Biology and Management of Mangroves, Honolulu, 1974. Vol. 1., Univ. of Florida, Gainesville, p. 3-22
Chapman, V. J. (1977). Ecosystems of the World. Vol. 1. Elsevier, Amsterdam

Chapman, V. J. (1984). Mangrove biogeography. In: Por, F. D., Dor, I. (Ed.) Hydrobiology of the Mangal. Dr. W. Junk Publishers, The Hague, p. 15-24

Dapaah-Siakwan, S. (1986). Simulation of regional groundwater flow with solute transport in the lower Athi-Tana Basin, Kenya. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

De Smedt, F., Bronders, J. (1985). A regional groundwater flow model based upon the variable source area concept. In: (Ed.) IWRA Vth World Congress on Water Resources - Water Resources for Rural Areas and their Communities. p. 511-521

Fennema, R. J., Neidrauer, C. J., Johnson, R. A., MacVicar, T. K., Perkins, W. A. (1994). A computer model to simulate natural Everglades hydrology. In: Davis, S. M., Ogden, J. C. (Ed.) Everglades, The Ecosystem and Its Restauration. St. Lucie Press, Delray Beach, p. 249-289

Holloway, M. (1994). Nurturing Nature. Sci. Am. 270 (4): 76-84

Isaac, W. E., Isaac, F. M. (1968). Marine botany of Kenya coast. 3: General account of environment, flora and vegetation. J. East Afr. Nat. Hist. Soc. Nat. Mus. 27: 7-28

Ituli, J. T. (1984). A regional groundwater flow model for the lower Athi-Tana catchment basin, Kenya. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Knutzen, J., Jasuund, E. (1979). Note on littoral algae from Mombasa, Kenya. J. East Afr. Nat. Hist. Soc. Nat. Mus. 168: 1-4

Macnae, W. (1968). A general account of the fauna and flora of mangrove swamps and forets in the Indo-West Pacific region. Adv. Mar. Biol. 6: 73-270

Mairson, A. (1994). The Everglades: Dying for Help. Nat. Geogr. 185 (4): 2-35

Ruwa, R. K. (1993). Zonation and distribution of creek and fringe mangroves in the semi-arid Kenyan coast. In: Lieth, H., Al Masoom, A. (Ed.) Towards the rational use of high salinity tolerant plants, Vol. 1. Kluwer Academic Publishers, The Hague, p. 97-105

Ruwa, R. K., Polk, P. (1986). Additional information on mangrove distribution in Kenya: some observations and remarks. Kenya J. Sci. Ser. B 7: 41-45

Snedaker, S. C. (1982). Mangrove species zonation: Why? In: Sen, D. N., Rasjpurohit, K. S. (Ed.) Tasks for Vegetation Science 2. Dr. W. Junk Publishers, The Hague, p. 111-125

Walsh, G. E. (1974). Mangroves: a review. In: Reinhold, R., Queen, W. (Ed.) Ecology of Halophytes. Academic press, New York, p. 51-74

Ecological differences in *Avicennia marina* (Forrsk.) Vierh. and *Rhizophora mucronata* Lam. in relation to groundwater flow along the Kenyan coast

Abstract

To establish the effects of groundwater flow along the Kenyan coast on the relative frequency, the mean maximum tree height, the above-ground biomass.m^{-2} and the number of propagules.m^{-2}, *Avicennia marina* and *Rhizophora mucronata* were studied in different mangrove areas along the Kenyan coast. Because of its double zonation *A. marina* was studied at the landwards and at the seawards end of the mangrove forest. High groundwater flow favours competitors of *A. marina* in the colonisation of the shore. Higher groundwater flow leads to mangrove trees with a higher mean maximum length and a higher above-ground biomass. The number of propagules.m^{-2} decreases with increasing groundwater flow. This is probably an effect of higher survival rates of the propagules in areas with a lower salinity. *A. marina* shows a number of adaptations to the dryer, more saline conditions on the land side of the mangrove forest.

Introduction

The mangrove ecosystem is both ecologically and economically extremely important for the tropical and subtropical coastal zone and its inhabitants. Mangrove forests filter land runoff, produce detritus contributing to offshore

productivity (Davis, 1940), are a shelter for numerous commercial and non-commercial fishes (Robertson and Duke, 1987, 1990) and are a home for many marine and terrestrial species. Local communities depend on the mangrove forest for fuelwood, charcoal, building poles (Kokwaro, 1985) and food (fish, crustaceans, molluscs) (Saenger *et al.*, 1983). More recently mangrove habitats are under increased pressure because of their use as disposal sites for human and industrial waste (Oteko, 1987).
Graham (1929) and Walter and Steiner (1936) were among the firsts to describe the East African mangrove ecosystem. More recently a number of publications concentrated on the description of the mangrove ecosystem along the Kenyan coast (Gallin *et al.*, 1989; Gang and Agatsiva, 1992; Van Speybroeck, 1992).
The distribution of mangroves along the Kenyan coast has been studied by Isaac and Isaac (1968), Kokwaro (1980, 1985) and, Ruwa and Polk (1986). Tack (1997) showed a relation between the distribution of mangrove ecosystems and areas with high groundwater flow along the Kenyan coast.
In this study we will present new data indicating a relation between a number of ecological variables of mangrove trees and groundwater flow in the coastal zone of Kenya.

Material and methods

Study sites

Fifteen mangrove forests or parts of them were chosen in function of the groundwater flow in their region. An overview of the study sites chosen is given in Table 1. Their location is also shown on the mangrove distribution map (Fig. 1).
Gallin *et al.* (1989) and Van Speybroeck (1992) described a topography-determined zonation pattern in Kenyan mangroves, with *Sonneratia alba* Sm. growing closest to the low water line, followed by *Rhizophora mucronata* Lam., *Bruguiera gymnorrhiza* (L.) Lam., *Ceriops tagal* (Perr.) C.B. Robinson, *Avicennia marina* (Forrsk.) Vierh., *Lumnitzera racemosa* Willd. and *Xylocarpus granatum* Koen.
The mangrove ecosystems in Kilifi district (Ngomeni, Mida and Kilifi) are dominated by *R. mucronata* and *C. tagal*. *A. marina* is common at Kilifi

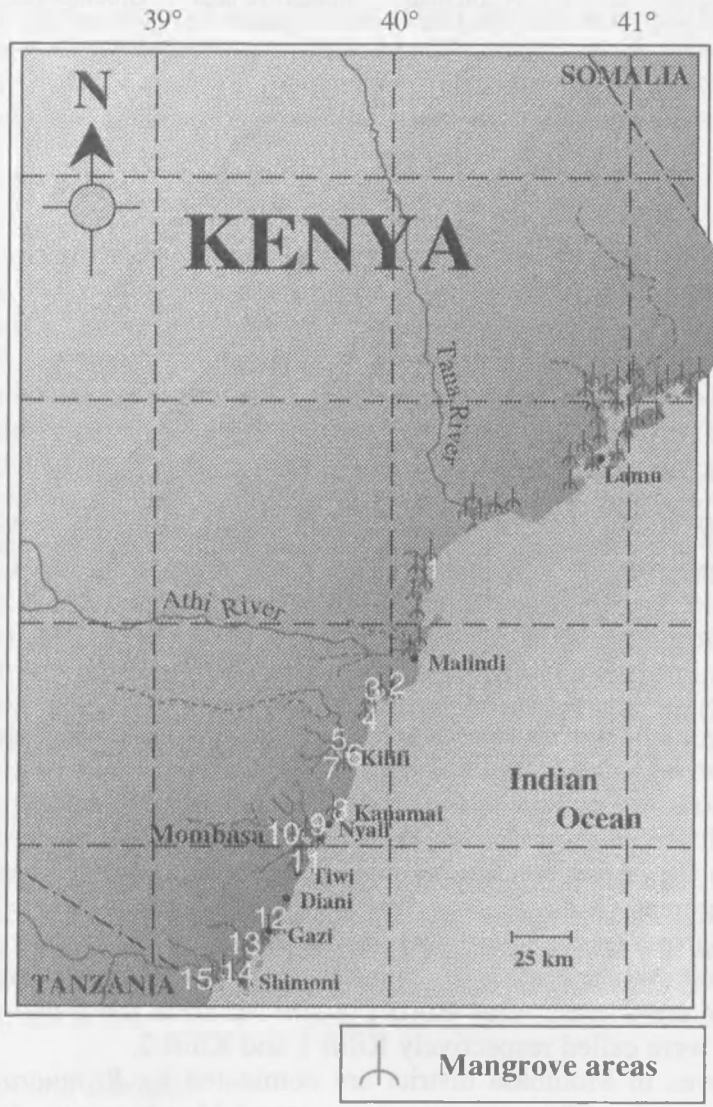

Figure 1. Mangrove distribution along the Kenyan coast.

Study site	N° on map (Fig. 1)	Mangrove area (Ha)	Groundwater flow ($m^2.day^{-1}$)
Ngomeni	1	1,815	6.57
Mida 1	2		3.59
Mida 2	3	1,600	4.00
Mida 3	4		3.69
Kilifi 1	5		2.54
Kilifi 2	6	360	2.95
Kilifi 3	7		4.29
Mtwapa	8	115	2.84
Mombasa 1 (Tudor Creek)	9	1,575	2.98
Mombasa 2 (Tudor Creek)	10	1,465	3.06
Mombasa 3 (Port Reitz)	11		2.03
Gazi	12	650	3.96
Ramisi + Funzi	13	2.715	3.73
Shimoni	14	4,265	3.49
Vanga	15		2.59

Table 1. An overview of the study sites with their respective number on the mangrove distribution map (Fig.1), their total mangrove area (ha) and the groundwater flow ($m^2.day^{-1}$) in their region (Tack, 1997).

Creek. At Ngomeni and Watamu (Mida Creek) a combination of *R. mucronata*, *B. gymnorrhiza* and *C. tagal* is dominating the forest. The other species occur localised. The mangrove forests of Mida and Kilifi were split in three parts with different groundwater flow (Tack, 1997). The mangrove area around Mida Creek was divided into mangroves bordering the islands in the centre of the creek (Mida 2), mangrove forest North of the creek (Mida 1) and the mangrove area south of the creek (Mida 3). The mangrove area in Kilifi was split in three parts in a similar way: the mangrove area around the mouth of the creek was called Kilifi 2 while the areas north and south of Kilifi Creek were called respectively Kilifi 1 and Kilifi 2.

The mangroves in Mombasa district are dominated by *R. mucronata*, *C. tagal* and *A. marina*. The mangrove area around Mombasa was divided in two parts: the area around Tudor Creek and the area around Port Reitz. The Tudor Creek area was divided in two major parts: Mombasa 1 includes the mangrove areas closest to the Indian Ocean, while Mombasa 2 includes the

mangrove areas around Tudor Creek more to the west.
The four study sites in Kwale district (Gazi, Ramisi + Funzi, Shimoni and, Vanga) are dominated by a combination of *R. mucronata*, *B. gymnorrhiza*, *X. granatum* and *C. tagal*. *S. alba* occurs towards the outer fringes towards the ocean, while *A. marina* dominates the areas where the rivers enter the ocean.

Relative frequency

In each of the study sites we studied the mangrove vegetation using four transects perpendicular to the mangrove zonation. Transects were chosen in those parts of the mangrove area with maximum growth (height) of *A. marina* and *R. mucronata*. The minimum distance between two transects was 500 m. 50 points along each transect have been studied using the point-centered quarter method (PCQM), described by Snedaker and Snedaker (1984). Using this methodology several variables can be calculated. However, within this study only the relative frequency of *A. marine* and *R. mucronata* was studied. The relative frequency of a species was defined as the absolute frequency of a species divided by the sum of frequencies of all species multiplied by 100 (%). Because of morphological differences between *A. marina* specimens growing in the landward and seaward side of the mangrove forest (De Bondt, 1995) both zones were studied separately. For each transect we calculated the mean relative frequency of respectively *A. marina* (landside), *A. marina* (seaside) and *R. mucronata*.

Mean Maximum Tree Height

The heights of *A. marina* (land and seaside) and *R. mucronata* occurring in a zone of 25 m on either side of the transects used in the PCQM were measured making use of a clipometre. For *A. marina* (seaside), *A. marina* (landside) and *R. mucronata* we calculated the mean height of the 25 largest trees in each transect.

Above-ground Biomass

Around each of the *R. mucronata* trees with maximum heights we established a plot measuring 20 by 20 m with the above mentioned *R. mucronata* tree in the centre of the plot. We measured the trunk circumference of all

trees in the plot on a height of 150 cm. The above-ground biomass of each individual tree was calculated from the best-fitting regression between trunk circumference and above-ground biomass: ln(biomass) =2.20 x ln(circumference at 150 cm height) - 7.81 (Slim *et al.*, 1996). For each transect we calculated the mean above-ground biomass.m^{-2}.

Propagules

In each transect 25 *A. marina* (seaside), 25 *A. marina* (landside) and 25 *R. mucronata* trees with maximum height were used to count the number of propagules on the tree per m^2. We calculated the mean number of propagules per m^2 for each transect.

Leafs

In each transect 25 *A. marina* (seaside), 25 *A. marina* (landside) and 25 *R. mucronata* trees with maximum height were used to measure 100 leaves on each tree. We measured length and width. Also leaf form, defined as the length width ratio, was calculated. In each transect we calculated the mean leaf length, mean leaf width and mean leaf form of *A. marina* (seaside), *A. marina* (landside) and *R. mucronata*.

Statistical analysis

In both *A. marina* (land and seaside) and *R. mucronata*, the relation between groundwater flow and the variables measured was analysed using Spearman Rank and Kendall τ correlation coefficients.
Differences between the land and seawards populations of *A. marina* were tested by a Mann-Whitney U test.

Results

Table 2 summarises the results of the non parametric Spearman R and Kendall τ tests for the different variables studied. Both Spearman R as Kendall τ correlation coefficients show significant negative correlations ($p<0.01$; $n=60$) between groundwater flow and the relative frequency of *A.*

Variables studied	Spearman R	Kendall τ
Groundwater flow - Relative frequency of *A. marina* (seaside)	0.06	0.04
Groundwater flow - Relative frequency of *A. marina* (landside)	-0.45*	-0.33*
Groundwater flow - Relative frequency of *R. mucronata*	0.70*	0.54*
Groundwater flow - Mean maximum height of *A. marina* (seaside)	0.63*	0.47*
Groundwater flow - Mean maximum height of *A. marina* (landside)	0.81*	0.65*
Groundwater flow - Mean maximum height of *R. mucronata*	0.36*	0.27*
Groundwater flow - Mean trunk circumference of *R. mucronata* at a height of 150 cm	0.33*	0.25*
Groundwater flow - Mean number of propagules.m^{-2} of *A. marina* (seaside)	-0.59*	-0.45*
Groundwater flow - Mean number of propagules.m^{-2} of *A. marina* (landside)	-0.41*	-0.30*
Groundwater flow - Mean number of propagules.m^{-2} of *R. mucronata*	-0.90*	-0.74*
Groundwater flow - Mean leaf length of *A. marina* (seaside)	0.16	0.11
Groundwater flow - Mean leaf length of *A. marina* (landside)	0.14	0.09
Groundwater flow - Mean leaf length of *R. mucronata*	0.04	0.03
Groundwater flow - Mean leaf width of *A. marina* (seaside)	0.01	0.00
Groundwater flow - Mean leaf width of *A. marina* (landside)	0.21	0.14
Groundwater flow - Mean leaf width of *R. mucronata*	0.02	0.01
Groundwater flow - Mean length/width ratio of *A. marina* leafs (seaside)	0.20	0.14
Groundwater flow - Mean length/width ratio of *A. marina* leafs (landside)	-0.20	-0.13
Groundwater flow - Mean length/width ratio of *R. mucronata* leafs	-0.01	-0.01

Table 2. Spearman Rank and Kendall τ correlation coefficients between groundwater flow and the variables studied (n=60). Significant differences ($p<0.01$) are marked with (*).

marina (landside) and the mean number of propagules.m^{-2} of respectively *A. marina* (seaside), *A. marina* (landside) and, *R. mucronata*. A significant positive correlation was found between groundwater flow and the relative frequency of *R. mucronata* and, the mean maximum height of respectively *A. marina* (seaside), *A. marina* (landside) and, *R. mucronata*.

The relative frequency of *A. marina* growing at the landside margins of the mangrove forest decreases with increasing groundwater flow. This is very clear in Figure 2. A groundwater flow of 2.03 $m^2.day^{-1}$ (Port Reitz) is good for a relative frequency between 11 and 21% while the relative frequency of *A. marina* (landside) drops to a third when groundwater reaches a flow of 6.57 $m^2.day^{-1}$ in Ngomeni. No differences are seen between the relative percentage of *A. marina* growing in the seaside part of the mangrove forest and groundwater flow.

The relative frequency of *R. mucronata* shows an approximately linear increase with increasing groundwater flow (Fig. 3). The relative frequency of *R. mucronata* is relative small (16-24%) at a groundwater flow of 2.03 $m^2.day^{-1}$ while it increases up to 40 to 60% in areas with a high groundwater outflow (6.57 $m^2.day^{-1}$).

Both the mean maximum heights of *A. marina* (Fig. 4) and *R. mucronata* (Fig. 5) increase with increasing groundwater flow. The mean maximum height of *A. marina* (landside) doubles between a groundwater flow of 2.03

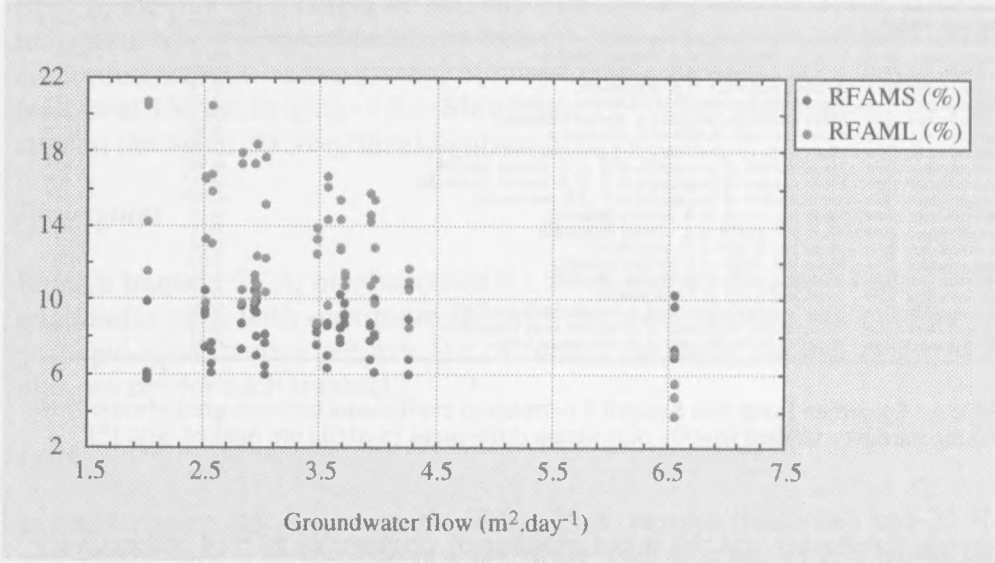

Figure 2. Relation between the relative frequencies (%) of respectively *A. marina* growing on the sea (RFAMS) and landside (RFAML) of the mangrove forest and the groundwater flow ($m^2.day^{-1}$).

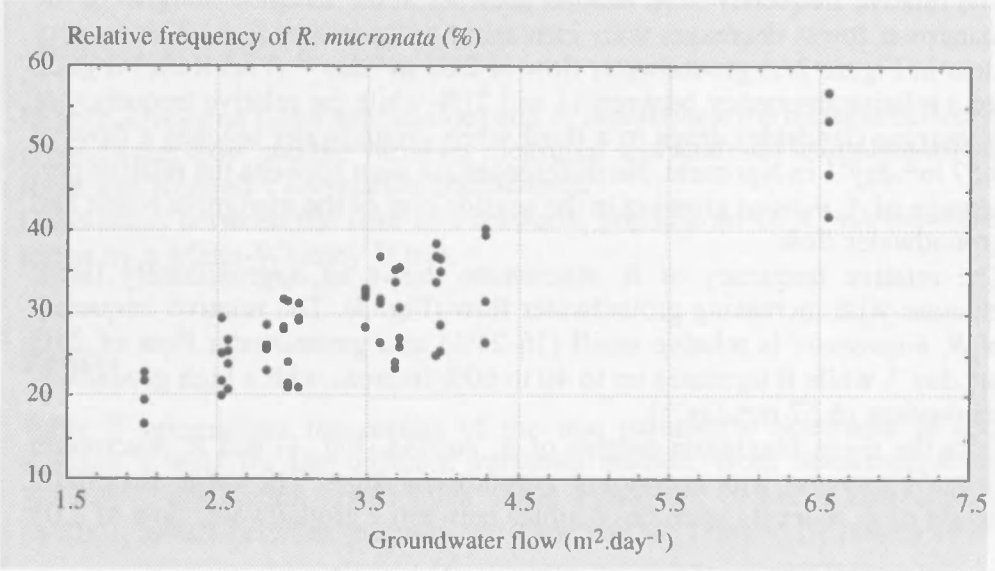

Figure 3. Relation between the relative frequencies (%) of *R. mucronata* and the groundwater flow ($m^2.day^{-1}$).

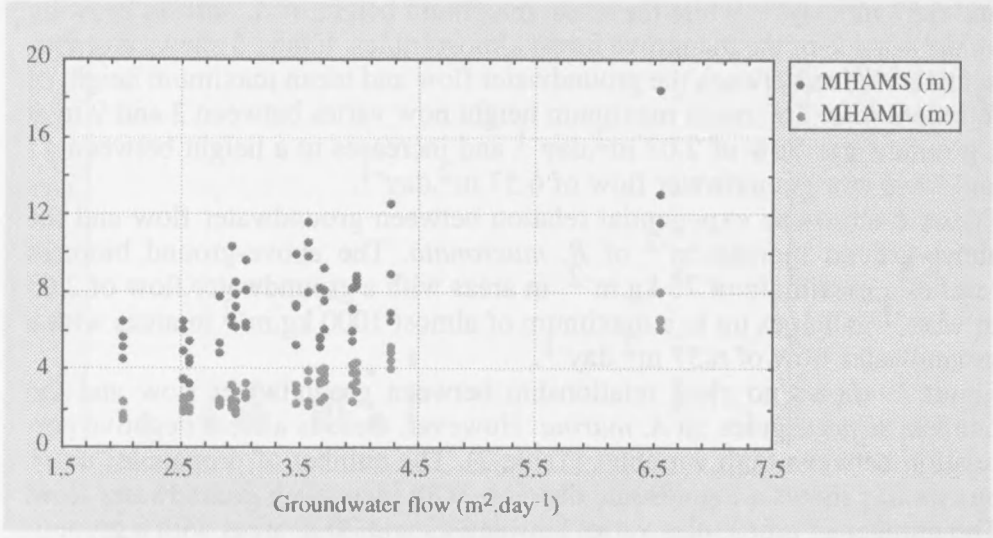

Figure 4. Relation between the mean maximum height (m) of respectively *A. marina* growing on the sea (MHAMS) and landside (MHAML) of the mangrove forest and the groundwater flow ($m^2.day^{-1}$).

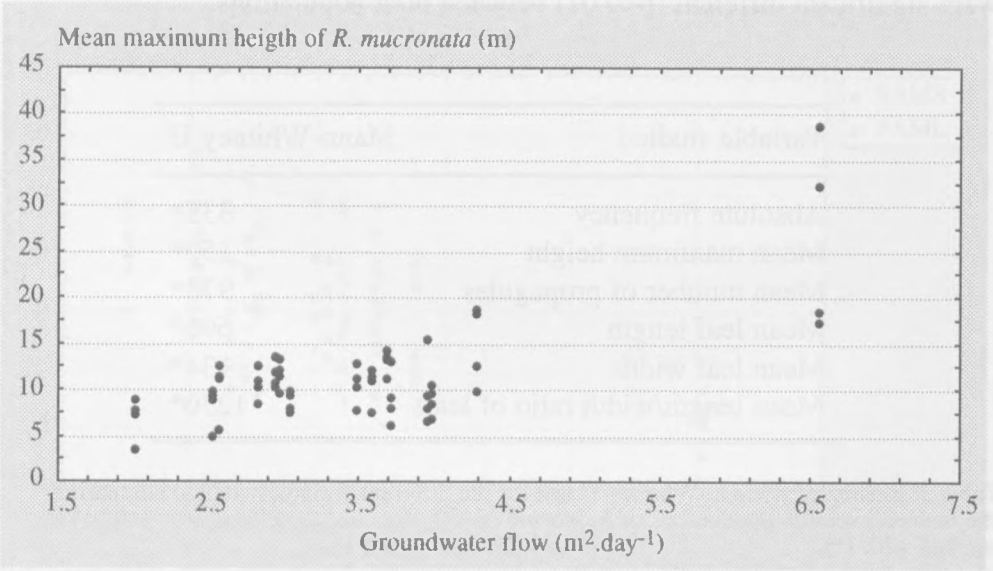

Figure 5. Relation between the mean maximum height (m) of *R. mucronata* and the groundwater flow ($m^2.day^{-1}$).

and 6.57 $m^2.day^{-1}$, while the mean maximum height of *A. marina* growing on the seaside of the mangrove forest almost triples. Figure 5 shows an exponential relation between the groundwater flow and mean maximum height of *R. mucronata*. The mean maximum height now varies between 3 and 9 m at a groundwater flow of 2.03 $m^2.day^{-1}$ and increases to a height between 17 and 39 m at a groundwater flow of 6.57 $m^2.day^{-1}$.

Figure 6 shows an exponential relation between groundwater flow and the above-ground biomass.m^{-2} of *R. mucronata*. The above-ground biomass reaches a maximum of 75 $kg.m^{-2}$ in areas with a groundwater flow of 2.03 $m^2.day^{-1}$ and goes up to a maximum of almost 1000 $kg.m^{-2}$ in areas with a groundwater flow of 6.57 $m^2.day^{-1}$.

Figure 7 shows no clear relationship between groundwater flow and the number of propagules on *A. marina*. However, there is a clear negative correlation between both variables (Table 2). The number of propagules on *R. mucronata* shows a logarithmic decrease with increasing groundwater flow. The number of propagules varies between 49 and 70 in areas with a groundwater flow of 2.03 $m^2.day^{-1}$ and between 7 and 9 in areas with a groundwater flow of 6.57 $m^2.day^{-1}$(Fig. 8).

Table 3 shows Mann-Whitney U values for the different variables studied when comparing the land and seaside populations of *A. marina*. All variables were significant different ($p<0.01$) between both populations.

Variable studied	Mann-Whitney U
Absolute frequency	335*
Mean maximum height	150*
Mean number of propagules	972*
Mean leaf length	696*
Mean leaf width	1134*
Mean length/width ratio of leafs	1230*

Table 3. Results of a Mann-Whitney U test for the different variables studied comparing the land and seaside populations of *A. marina* (n=60). Significant differences ($p<0.01$) are marked with (*).

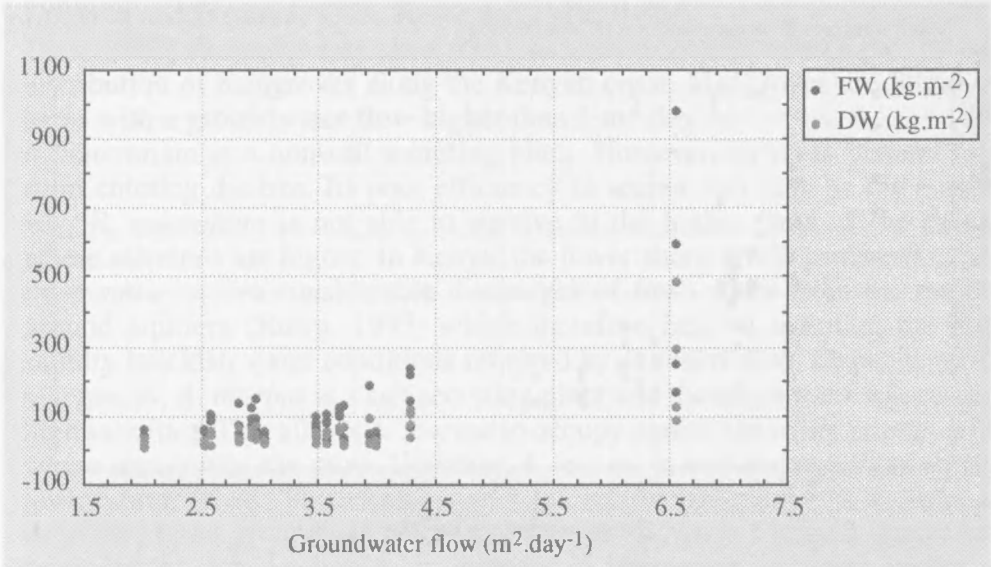

Figure 6. Relation between the mean above-ground biomass of *R. mucronata* in respectively kg fresh weight.m^{-2} (FW) and in kg dry weight.m^{-2} (DW) and the groundwater flow (m^2.day^{-1}). Conversion factor used to calculate dry weight (DW) from estimates of fresh weight (FW) is calculated by Slim *et al.* (1996).

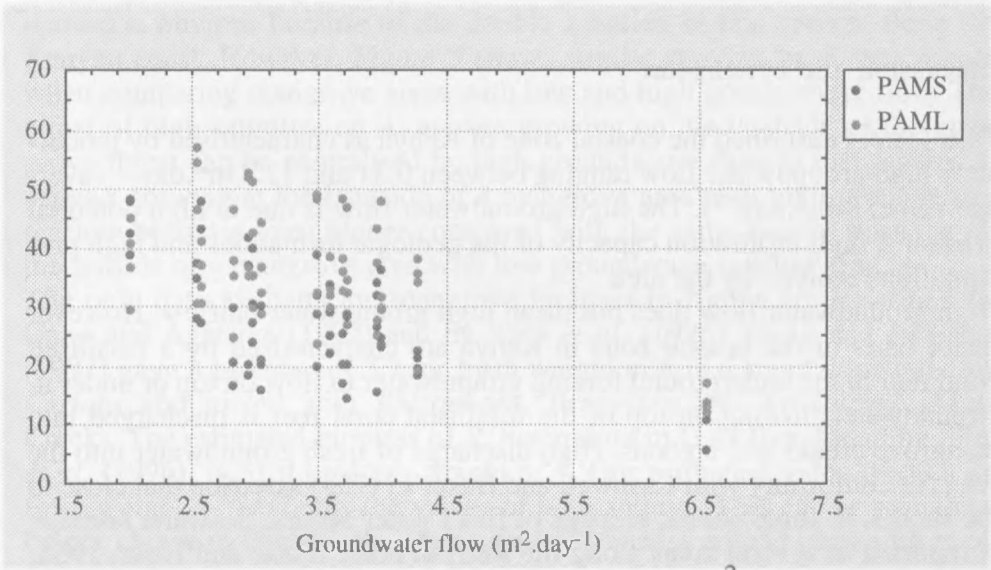

Figure 7. Relation between the mean number of propagules per m^2 of respectively *A. marina* growing on the sea (PAMS) and landside (PAML) of the mangrove forest and the groundwater flow (m^2.day^{-1}).

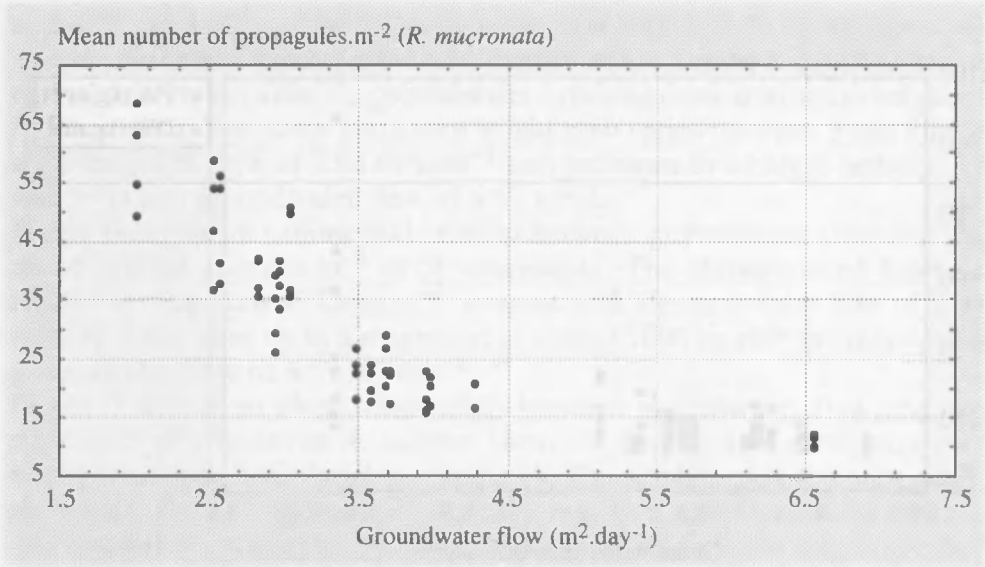

Figure 8. Relation between the mean number of propagules.m^2 of *R. mucronata* and the groundwater flow (m^2.day^{-1})

Discussion and conclusion

Tack (1997) described the coastal zone of Kenya as characterised by moderately high groundwater flow ranging between 0.31 and 12.8 m^2.day^{-1} (average value: 4 m^2.day^{-1}). The high groundwater flow is due to high potential gradients, high infiltration capacity of the geologic formations and high precipitation received by the area.

High groundwater flow does not mean high groundwater outflow. However major parts of the coastal zone in Kenya are characterised by a fossilised coral reef in the underground forcing groundwater to flow on top or under it. Groundwater flowing on top of the fossilised coral reef is discharged into mangrove creeks and lagoons. High discharge of fresh groundwater into the sea is in conformity with Cashwell and Baker's (1953) assertion that close to the shores, in some points, seepage of fresh water occurs. Seawater seepage is reported in several areas along the Kenyan coast (Isaac and Isaac, 1968;

Knutzen and Jasuund, 1979; Ruwa and Polk, 1986).
Tack (1997) showed a very clear relation between groundwater flow and the distribution of mangroves along the Kenyan coast. Mangroves are found in areas with a groundwater flow higher than 1 $m^2.day^{-1}$.
R. mucronata is a non-salt secreting plant. However, its roots prevent salt from entering the tree. Its poor efficiency to secrete salt may be the reason why *R. mucronata* is not able to survive in the higher parts of the shore, where salinities are higher. In Kenya, the lower shore levels preferred by *R. mucronata* receive considerable discharges of fresh water from the underground aquifers (Ruwa, 1993) which therefore help to maintain the low salinity brackish water conditions required by *R. mucronata*. Opposite to *R. mucronata*, *A. marina* is a salt secreting plant and therefore more tolerant to high salinities. This allows *A. marina* to occupy almost the entire coastal area where mangroves can grow. However, *A. marina* is a poor competitor on the lower shore levels. This explains why the relative frequency of *A. marina* decreases when groundwater flow increases as shown in Figure 2. Increased groundwater flow leading to an increase in freshwater discharge makes it easier for the competitors of *A. marina* (e.g. *R. mucronata*, Fig. 3) to colonise larger parts of the shore.
The stress factor caused by salinity gradients across the shore may be illustrated by the stunting of *A. marina* at higher shore levels. Stunting by *A. marina* is obvious because of the double zonation of this species along the Kenyan coast. However, Figure 5 shows similar stunting by *R. mucronata* when comparing mangrove areas with low and high groundwater flow. The effect of high salinities on *A. marina* growing on the landside of the mangrove forest can be neutralised by high groundwater flow in that region. *A. marina* growing at the landside of a mangrove area with high groundwater outflow becomes even higher compared with the same species growing on the seaside of a mangrove area with low groundwater outflow (Fig. 4).
The only data available on mangrove biomass in Kenya are described by Gang and Agatsiva (1992) and by Slim *et al.* (1996). Gang and Agatsiva (1992) gave a biomass of 3.7 kg fresh $weight.m^{-2}$ in a stand dominated by *Ceriops* and mixed with *Rhizophora*, *Bruguiera* and *Xylocarpus* (Mida Creek). The estimated biomass of *R. mucronata* in Gazi Bay, found by Slim *et al.* (1996), is 51.0 kg fresh $weight.m^{-2}$. Our estimated value of 68.6 kg fresh $weight.m^{-2}$ exceeds the values of both authors. This can be explained by our choice to measure the above-ground biomass around trees with max-

imum heights. Putz and Chan (1986) summarise some of the results obtained by other authors. They describe a biomass increase from 1.6-5.0 kg dry weight.m^{-2} for a 5 year old forest to 27.0-47.4 kg dry weight.m^{-2} for forests over 28 years. Our estimate of 68.6 kg fresh weight.m^{-2}, equivalent to 33.5 kg dry weight.m^{-2} (Slim *et al.,* 1996) indicates that our plots lay in fully grown forest. Saenger and Snedaker (1993) showed a relation between latitude, stand height and above-ground biomass. Slim *et al.* (1996) applied this relation to Gazi Bay, which is at 4°25'S, giving an expected biomass of 21 kg dry weight.m^{-2} for *R. mucronata.* This value is comparable with the values found in Figure 6: 13.7 kg dry weight.m^{-2} in areas with a groundwater flow of 2.03 m^2.day^{-1} (Mombasa, Port Reitz) and 240.1 kg dry weight.m^{-2} in areas with a groundwater flow of 6.57 m^2.day^{-1} (Ngomeni). Those figures indicate the necessity to include groundwater flow data into biomass estimations of mangrove forests.

An increase in groundwater flow is related with a decrease in the number of propagules.m^{-2}. This is the case by as well *A. marina* (Fig. 7) as *R. mucronata* (Fig. 8). No references were found comparing the number of propagules between different mangrove areas in Kenya. Kairo (1995) and Verneirt (1994) showed a higher mortality of mangrove propagules in the more elevated parts of the mangrove area studied. So there is a possibility that the survival rate of mangrove propagules increases with a decrease in salinity. This hypothesis can also explain the decreasing number of propagules in function of an increase in groundwater flow.

De Bondt (1995) studied the double zonation of *A. marina* in Gazi Bay (Kenya). *A. marina* growing on the landside of the mangrove forest is smaller, has less but larger pneumatophores, has smaller leaf lengths and widths and has more but smaller stomata. Our data (Fig. 3) show similar results. Those differences are adaptations to the dryer, more saline conditions on the land side of the mangrove forest and are comparable with differences noticed between mangrove areas with high and low groundwater flow.

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References

Cashwell, P. V., Baker, B. H. (1953). Geology of the Mombasa-Kwale Area. Bulletin Geol. Surv. Kenya, Report No. 24, Nairobi

Davis, J. H. (1940). The ecology and geological role of mangroves in Florida. Carnegie Inst. Wash. Publ. 517: 305-412

De Bondt, R. (1995). Het disjuncte zonatiepatroon van *Avicennia marina* (Forsk.) Vierh. langs de Oostkust van Afrika (Kenya, Gazi Bay). Een ecologisch-vergelijkende studie. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Gallin, E., Coppejans, E., Beeckman, H. (1989). The mangrove vegetation of Gazi Bay. Bull. Soc. r. Bot. Belg. 122: 197-207

Gang, P. O., Agatsiva, J. L. (1992). The current status of mangroves along the Kenyan coast: a case study of Mida Creek mangroves based on remote sensing. Hydrobiologia 247: 29-36

Graham, R. M. (1929). Notes on the mangrove swamps of Kenya. J. E. Afr. U. Nat. Hist. Soc. 36: 157-164

Isaac, W. M. E., Isaac, F. M. N. (1968). Marine botany of the Kenya coast. 3. General account of the environment, flora and vegetation. J. E. Afr. Nat. Hist. Soc. 27: 1-28

Kairo, J. G. (1995). Community Participatory Forestry for Rehabilitation of Deforested Mangrove Areas of Gazi Bay (Kenya). Final Technical Report, University of Nairobi, Nairobi

Knutzen, J., Jasuund, E. (1979). Note on littoral algae from Mombasa, Kenya. J. East Afr. Nat. Hist. Soc. Nat. Mus. 168: 1-4

Kokwaro, J. O. (1980). Economic importance and local use of the Kenyan mangroves. In: (Ed.) Proceedings of the Kenya National Seminar on Agroforestry. ICRAF, Nairobi, p. 377-386

Kokwaro, J. O. (1985). The distribution and economic importance of the mangrove forests in Kenya. J. E. Afr. Nat. Hist. Soc. 75: 1-12

Oteko, D. (1987). Analysis of some major and trace metals in sediments of Gazi, Makupa and Tudor Creeks of the Kenya coast. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Putz, F. E., Chan, H. T. (1986). Tree growth, dynamics, and productivity in a mature mangrove forest in Malaysia. For. Ecol. Manag. 17: 211-230

Robertson, A. I., Duke, N. C. (1987). Mangroves as nursery sites: comparisons of the abundance and species composition of fish and crustaceans in mangrove and other nearshore habitats in tropical Australia. Mar. Biol. (Berlin) 96: 193-205

Robertson, A. I., Duke, N. C. (1990). Recruitment, growth and residence time of fishes in a tropical mangrove ecosystem. Estuar. Coast. Shelf Sci. 31: 723-43

Ruwa, R. K. (1993). Zonation and distribution of creek and fringe mangroves in the semi-arid Kenyan coast. In: Lieth, H., Al Masoom, A. (Ed.) Towards the rational use of high salinity tolerant plants, Vol. 1. Kluwer Academic Publishers, The Hague, p. 97-105

Ruwa, R. K., Polk, P. (1986). Additional information on mangrove distribution in Kenya: some observations and remarks. Kenya J. Sci. Ser. B 7: 41-45

Saenger, P., Hegerl, E. J., Davie, J. D. S. (1983). Global status of mangrove ecosystems. Environmentalist (Suppl.) 3: 1-88

Saenger, P., Snedaker, S. C. (1993). Pantropical trends in mangrove above-ground biomass and annual litterfall. Oecologia 96: 293-299

Slim, F. J., Gwada, P. M., Kodjo, M., Hemminga, M. A. (1996). Biomass and Litterfall of *Ceriops tagal* and *Rhizophora mucronata* in the Mangrove Forest of Gazi Bay, Kenya. Mar. Freshw. Res. 47: 999-1007

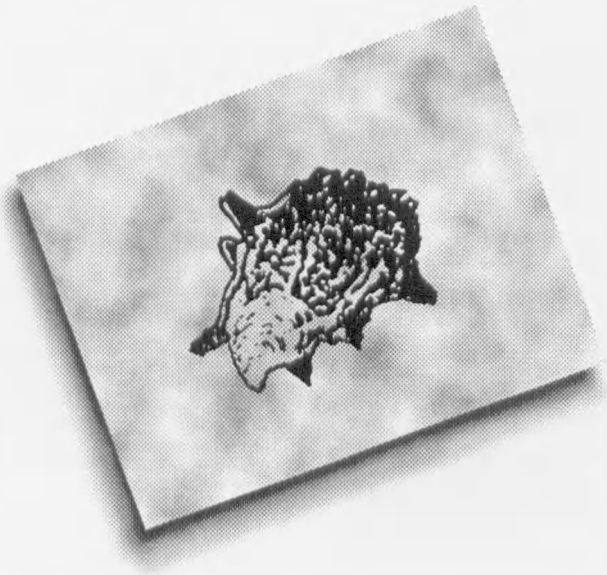
Snedaker, S. C., Snedaker, J. G. (1984). Monographs on oceanic methodology. 8. The mangrove ecosystem: research methods. UNESCO, Paris

Tack, J. F. (1997). Groundwater flow in the coastal zone influences mangrove distribution. In: Behavioural aspects of the mangrove oyster *Saccostrea cucullata* (von Born, 1778) explaining its macro and micro distribution along the Kenyan coast. Unpublished Ph.D. thesis, Vrije Universiteit Brussel, Brussels, p. 57-79

Van Speybroeck, D. (1992). Regeneration strategy of mangroves along the Kenya Coast: a first approach. Hydrobiologia 247: 243-251

Verneirt, M. (1994). Faunistische factoren in de regeneratie van de Oostafrikaanse mangrove. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Walter, H., Steiner, M. (1936). Die Ökologie der ost-afrikanischen Mangroven. Zeits. Bot. 30: 5-193



Chapter

4

FEEDING BEHAVIOUR

The uptake of colloidal melanin from sea water by marine bivalves

Abstract

A number of coastal organisms (e.g. bivalves) are known as important consumers of particulate organic carbon (POC) in the sea. In this paper we present new evidence that they also are important consumers of 'dissolved' organic carbon (DOC) in the colloidal size range down to about 0.2 µm in diameter. Those colloids have an important role in the global flux of carbon in the seas.

We compare the uptake of colloidal DOC by marine bivalves of the North Sea and of the western Indian Ocean.

To measure this uptake we made use of colloidal melanin as an alternative way to measure the uptake of colloidal DOC.

Problem

Dissolved organic carbon (DOC) can be a substantial source of nutrition for marine organisms (STEPHENS, 1968).

Small phytoplankton and bacteria often dominate primary production of dissolved and particulate organic matter in marine ecosystems. This has led to increased interest in the feeding biology of mucous-net suspension feeders. It has been assumed that they consume particles of this size efficiently (MULLIN, 1983).

Oikopleurids are appendicularian tunicates, often occurring in high densities in discrete strata at various depths, and may under such conditions clear 30-60% of the water mass in 24 hours (ALLDREDGE, 1981). Obviously, they

may remove and repack colloidal DOC (>0.2 µm particle size) rapidly under such conditions (FLOOD *et al.*, 1992). On the basis of filter parameters this ability to graze on colloidal DOC is probably shared by caddisfly larvae (WALLACE & MALAS, 1976), pedal worms (FLOOD & FIALA-MEDIONI, 1982), ascidians (FLOOD & FIALA-MEDIONI, 1981), salps (BONE *et al.*, 1991) and amphioxus (FLOOD, 1981).
WRIGHT (1982) described the nutritional role of amino acids in filter-feeding marine invertebrates. MANAHAN (1983) studied the role of dissolved organic material in the nutrition of pelagic larvae. MANAHAN *et al.* (1982) demonstrated the net uptake of dissolved amino acids from natural seawater by the mussel *Mytilus edulis*.
In this study we will examine the uptake by bivalves of 'dissolved' organic carbon (DOC) in the colloidal size range down to about 0.2 µm in diameter. Such colloids are a more significant portion of the dissolved organic material in the sea than previously believed (KOIKE *et al.*, 1990). They have an important role in the global flux of carbon.

Material and methods

Observations were made on 13 species of bivalves. Of each species we collected six specimens. Six species of bivalves were collected in a tropical environment (Indian Ocean), seven species in a non-tropical region (North Sea).
The six tropical species were collected in the neighbourhood of Gazi, a small village 49 km south of Mombasa (Kenya). Of those six species two were found in a mangrove area (*Saccostrea cucullata* and *Modiolus tulipa*), three were found in the seagrass meadows (*Codalia punctata*, *Trachycardium flavum* and *Tellina staurella*) and two were found on the coral reefs (*Tridacna maxima* and *Tellina staurella*). We have to mention the presence of symbiotic algae (zooxanthellae) in the mantle lobe of *T. maxima*. When the number of zooxanthellae in the mantle lobe is too high, *T. maxima* will ingest large numbers of the algae. Whenever the number of zooxanthellae in the mantle lobe is normal, *T. maxima* will behave as the other filter feeders used in this study.
The seven non-tropical species were collected in the North Sea in front of the Belgian and the Dutch coast (Zeeuws Vlaanderen). We can separate them in

two groups. Those found in the littoral zone (*Crassostrea gigas*, *Mytilus edulis* and *Cerastoderma (Cardium) edule*) and those found in the sublittoral zone (*Ostrea edulis*, *Pecten maximus*, *Astarte sulcata* and *Modiolus modiolus*).

Seawater prefiltered through a 0.4 µm membrane filter contains about 3.6 $mg.l^{-1}$ DOC. Most of this DOC is biodegradable polymers of relative molecular mass 4,000-100,000 (FLOOD *et al.*, 1992). Larger colloidal particles, up to 1 µm in diameter, account for at least 10% of additional DOC (KOIKE *et al.*, 1990). To examine whether bivalves are able to consume colloidal DOC we offered suspensions of colloidal melanin from the ink sac of the cuttlefish *Sepia sp.* to each of the bivalve species. Colloidal melanin was chosen because of its easy visibility, high stability (BAGNARA & HADLEY, 1973) and lack of tendency to agglutinate (FLOOD *et al.*, 1990).
We offered highly diluted *Sepia* ink to six adult bivalves of each species (tropical and non-tropical). The animals were placed in separate 600 ml PVC jars filled with seawater taken at the animals original habitat. *Sepia* ink, prefiltered through a Whatman GF/C glass fibre filter (1.2 µm nominal pore size), was added in concentrations of 0.5-7 $mg.l^{-1}$. Those concentrations are covering much of the range of naturally occurring dissolved organic matter in the sea (FLOOD *et al.*, 1992).
Removal of colloidal *Sepia* ink from the seawater by the several bivalves was measured as the change in absorbence of 500 nm light using a spectrophotometer. Conversion to dry weight was done using dilution series made from a known mass of ink (Fig. 1). Spectrophotometric measurements were done with an one hour interval.

Results

Figure 1 shows a linear correlation (r^2=0.972, n=13, $p \leq 10^{-4}$) between the absorbence of 500 nm light and the dry weight of melanin dissolved in one litre of seawater. Differences in absorbence indicate differences in melanin concentration in the seawater.
We found that all bivalve species remove *Sepia* ink at specific rates. Differences in uptake of dissolved organic matter between specimens of the

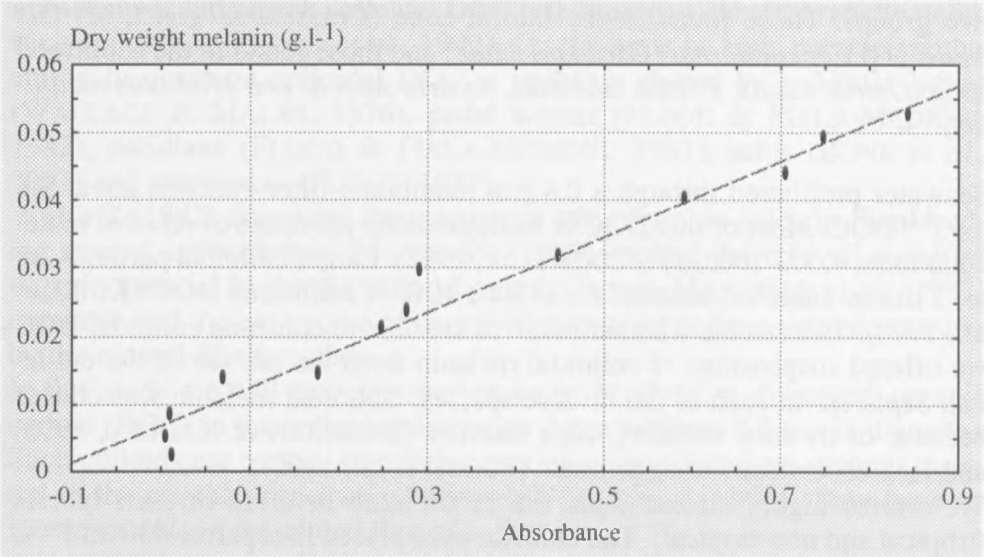


Figure 1. Linear correlation ($y=0.056x+0.0067$, $r^2=0.972$, $n=13$, $p\leq10^{-4}$) between the absorbence of 500 nm light and the dry weight of melanin dissolved in one litre of seawater.

same species are related to the dry weight of the bivalves. Similar results were found for the tropical and non-tropical bivalve species.

The rates of colloidal melanin removal for the different bivalve species are given in Table 1. This table shows a clear difference in colloidal melanin removal between the tropical and the non-tropical species except in the cases of *Tridacna maxima* and *Tellina staurella*, both found on the coral reef. The tropical species found in the mangrove and seagrass ecosystem have a rate of colloidal melanin removal two to three times higher than the species found in non-tropical regions.

The first part of Table 1 shows a clear difference between the colloidal melanin uptake of the coral reef species on one side and the mangrove and seagrass meadow species on the other side. We find a significant difference ($p\leq0.05$) between the two groups. There is no significant difference between the bivalves found in the mangrove area and those found in the seagrass meadows area.

species	rate of colloidal melanin removal ± SD [mg.l^{-1}.h^{-1}]
tropical species	
Saccostrea cucullata	0.55 ± 0.03
Modiolus tulipa	0.57 ± 0.02
Codalia punctata	0.52 ± 0.02
Trachycardium flavum	0.51 ± 0.02
Tellina staurella (seagrass meadows)	0.52 ± 0.02
Tridacna maxima	0.14 ± 0.03
Tellina staurella (coral reef)	0.13 ± 0.01
non-tropical species	
Crassostrea gigas	0.23 ± 0.01
Mytilus edulis	0.21 ± 0.01
Cerastoderma (Cardium) edule	0.22 ± 0.01
Ostrea edulis	0.18 ± 0.01
Pecten maximus	0.16 ± 0.01
Astarte sulcata	0.16 ± 0.01
Modiolus modiolus	0.17 ± 0.01

Table 1. Rates of colloidal melanin removal [mg.l^{-1}.h^{-1}] for the different bivalve species used in this study.

The second part of Table 1 shows more or less the same situation for the non-tropical bivalves. Here we see a significant difference ($p \leq 0.05$) in colloidal melanin uptake between the littoral and the sublittoral species. However the difference is much smaller compared with the difference noticed in the first part of Table 1 between the coral reef species and the mangrove and seagrass meadow species.

Discussion

There is a clear difference in uptake of colloidal melanin between the tropical species examined. The uptake of species collected in the mangroves (*Saccostrea cucullata* and *Modiolus tulipa*) and in the seagrass meadows (*Codalia punctata*, *Trachycardium flavum* and *Tellina staurella*) is signifi-

cant higher than the species collected on the coral reef (*Tridacna maxima* and *Tellina staurella*). The difference in melanin uptake is very clear in *Tellina staurella*. This species was collected as well in the seagrass meadows as on the coral reef. The specimens of both locations are taking up colloidal melanin at significantly different rates ($p \leq 0.05$). This indicates that the differences in uptake can not be explained by the choice of the different species. However this can be part of the problem.

HEMMINGA *et al.* (1994) investigated carbon fluxes in Gazi Bay (Kenya). They showed that, as far as particulate organic matter (POM) fluxes are concerned, the mangrove forest and the adjacent seagrass meadows are tightly coupled, but that the nearby coral reef may exist in relative isolation. Our data give the impression that such a relationship could also exist for dissolved organic material (DOC).

RODELLI *et al.* (1984) determined the carbon isotope signature of a large number of organisms in a coastal area with mangroves. They found evidence that some animals from the mangroves and nearby mudflats and inlets derived a part of their nutrition from mangrove detritus, but that this was not the case for animals collected further offshore (>2 km). In the studies of ALONGI *et al.* (1989), ALONGI (1990) and RODELLI *et al.* (1984) the poor nutritional quality of the outwelled material is frequently mentioned. This poor nutritional value of the particulate material in the mangrove and seagrass meadow areas can be an explanation why marine organisms, living in this region, are seeking solace in the uptake of dissolved organic carbon.

Table 1 shows that there is also a difference in the colloidal melanin uptake of littoral and sublittoral species found in the North Sea. Knowing that the surface waters of the North Sea are very productive the above hypothesis for the tropical species seems not to be valid to explain the differences in colloidal melanin uptake of *Crassostrea gigas*, *Mytilus edulis* and *Cerastoderma (Cardium) edule* on the one end and *Ostrea edulis, Pecten maximus*, *Astarte sulcata* and *Modiolis modiolis* on the other hand. SUZUKI *et al.* (1985) and SUGIMURA & SUZUKI (1988) have unveiled a new method for measuring the total concentrations of DOC in seawater. They report that the concentrations of dissolved organic carbon are about four times higher in surface waters and two times higher in deep water than previously thought. They also show that DOC concentrations decrease strongly with depth, mirroring the increases in dissolved inorganic carbon. The higher amounts of DOC in surface waters can explain why more DOC is taken up by *C. gigas*,

M. edulis and *C. edule*, compared with *O. edulis*, *P. maximus*, *A. sulcata* and *M. modiolis* living in deeper water. This can also explain why *O. edulis* has a relative high uptake of colloidal melanin compared with the other sublittoral species.

If we compare the tropical and non-tropical species we see that the uptake of colloidal melanin is comparable except for those species found in the mangrove and seagrass meadow area. The low nutritional value of the POM in this region (ALONGI *et al.*, 1989; ALONGI, 1990 and RODELLI *et al.*, 1984) can be an explanation for this phenomenon.

The colloidal ink particles were ingested by all bivalve species as evidenced from their blackened gut contents. There was no sign of digestion or cellular uptake of ink particles in accordance with the high stability of naturally occurring melanin biopolymers (BAGNARA & HADLEY, 1973). However, making use of cell-free filtrates of algal cultures, we have preliminary evidence of ingestion and subsequent digestion of more easily biodegradable colloidal DOC by *S. cucullata*.

It is difficult to relate our results to actual removal rates of bivalves in the sea. The size distribution and the shape of naturally occurring colloidal particles, as well as their overall quantity as a subfraction of DOC, is only partly known (KOIKE *et al.*, 1990 and FLOOD, 1978). A conservative estimate of 20% of DOC as grazable by bivalves, means that this source of food is as important for bivalves as is total POC.

The present study found a significant difference in uptake of ink colloidals between several species of tropical bivalves. Coral reef species showed a low uptake while mangrove and seagrass meadow species showed a high uptake rate. In the non-tropical species from the North Sea a remarkable difference was observed between littoral and sublittoral species. It was shown that species inhabiting shallow waters exhibited higher rates of uptake potentially due to higher DOC concentrations in surface waters.

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References

ALLDREDGE, A.L., 1981: The impact of appendicularian grazing on natural food concentrations in situ. Limnol. Oceanogr., **26**(2): 247-257.

ALONGI, D.M., 1990: Abundances of benthic microfauna in relation to outwelling of mangrove detritus in a tropical coastal region. Mar. Ecol. Prog. Ser., **63**: 53-63.

ALONGI, D.M., K.G. BOTO & F. TIRENDI, 1989: Effect of exported mangrove litter on bacterial productivity and dissolved organic carbon fluxes in adjacent tropical nearshore sediments. Mar. Ecol. Prog. Ser., **56**: 133-144.

BAGNARA, J.T. & M.E. HADLEY, 1973: Chromatophores and color change, the comparative physiology of animal pigmentation. Prentice-Hall, Englelwood Cliffs, NJ; 46-50.

BONE, Q., J.-C. BRACONNOT & K.P. RYAN, 1991: On the pharyngeal feeding filter of the salp *Pegea confoederata* (Tunicata: Thaliacea). Acta Zool. Stockh., **72**: 55-60.

FLOOD, P.R., 1978: Filter characteristics of appendicularian food catching nets. Experientia, **34**: 173-175.

FLOOD, P.R., 1981: On the ultrastructure of mucus. Biomed. Res. Suppl., **2**: 49-53.

FLOOD, P.R., D. DEIBEL & C.C. MORRIS, 1990: Visualization of the transparent gelatinous house of the pelagic tunicate *Oikopleura vanhoeffeni* using *Sepia* ink. Biol. Bull. mar. biol. Lab. Woods Hole, **178**: 118-125.

FLOOD, P.R., D. DEIBEL & C.C. MORRIS, 1992: Filtration of colloidal melanin from sea water by planktonic tunicates. Science, **355**: 630-632.

FLOOD, P.R. & A. FIALA-MEDIONI, 1981: Ultrastructure and Histochemistry of the Food Trapping Mucous Film in Benthic Filter-Feeders (Ascidians). Acta Zool. Stockh., **62**: 53-65.

FLOOD, P.R. & A. FIALA-MEDIONI, 1982: Structure of the Mucous Feeding Filter of *Chaetopterus variopedatus* (Polychaeta). Mar. Biol., **72**: 27-33.

HEMMINGA, M.A., F.J. SLIM, J. KAZUNGU, G.M. GANSSEN, J. NIEUWENHUIZE & N.M. KRUYT, 1994: Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). Mar. Ecol. Prog. Ser., **106**: 291-301.

KOIKE, I., H. SHIGEMITSU, T. KAZUKI & K. KAZUHIRO, 1990: Role of sub-micrometre particles in the ocean. Science, **345**: 242-244.

MANAHAN, D.T., 1983: The uptake and metabolism of dissolved amino acids by bivalve larvae. Biol. Bull., **164**: 236-250.

MANAHAN, D.T., S.H. WRIGHT, G.C. STEPHENS & M.A. RICE, 1982: Transport of dissolved amino acids by the mussel, *Mytilus edulis*: demonstration of net uptake from natural seawater. Science, **215**: 1253-1255.

MULLIN, M.M., 1983: *In situ* measurements of filtering rates of the salp *Thalia democratica*, on phytoplankton and bacteria. J. Plankton Res., **5**: 279-289.

RODELLI, M.R., J.N. GEARING, P.J. GEARING, N. MARSHALL & A. SASEKUMAR, 1984: Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems.

Oecologia, **61**: 326-333.
STEPHENS, G.C., 1968: Dissolved organic matter as a potential source of nutrition for marine organisms. Am. Zool., **8**: 95-106.
SUGIMURA, Y. & Y. SUZUKI, 1988: A high temperature catalytic oxidation method of non-volatile dissolved organic carbon in seawater by direct injection of liquid sample. Marine Chem., **24**: 105-131.
SUZUKI, Y., Y. SUGIMURA & T. ITOH, 1985: A catalytic oxidation method for the determination of total nitrogen dissolved in seawater. Marine Chem., **16**: 83-97.
WALLACE, B.J. & D. MALAS, 1976: The significance of the elongate rectangular mesh found in capture nets of fine particle filter feeding trichoptera larvae. Arch. Hydrobiol., **77**: 205-212.
WRIGHT, S.H., 1982: A nutritional role for amino acid transport in filter-feeding marine invertebrates. Am. Zool., **22**: 621-634.

The feeding behaviour of *Saccostrea cucullata* (von Born, 1778): the role of particulate and dissolved organic carbon

Abstract

To study the feeding behaviour of *S. cucullata* (von Born, 1778) the uptake and clearance rates of particulate and dissolved organic carbon have been measured as a function of its submersion time. *S. cucullata* takes up large amounts of DOC in the size range 0.2 to 1.2 µm in the first 2 hours the oyster is submerged. The most important part of this DOC consists of colloidal particles and bacteria. *S. cucullata* shows a clear preference to feed on bacteria. However, the large concentrations of colloidal particles makes this food source to the most important one of the mangrove oyster. The colloidal material shows a comparable $\delta^{13}C$ value with the $\delta^{13}C$ values of seagrasses. Between the third and the fourth hour *S. cucullata* shifts from a preferential clearance of the DOC component towards a preferential clearance of the POC component of the food. Besides phyto and zooplankton *S. cucullata* takes up large seston particles originating from mangrove detritus. The uptake of POC particles is related to its diameter. The longer the submersion time the greater the diameter of the particles taken up by *S. cucullata*.

Introduction

Saccostrea cucullata (von Born, 1778) is an edible oyster found in the upper littoral zone, following the terminology of Lewis (1964) and Hartnoll

(1976). It is found on the trunks and stilt roots of mangrove plants and rocky substrata in brakish marine environments. Day (1974) noted the zoogeographical distribution of this species in the western part of the Indian Ocean. Ecological studies of this species have been carried out over the entire region: Seychelles (Taylor, 1968), Aldabra (Taylor, 1970), Tanzania (Hartnoll, 1976), Somalia (Chelazzi and Vannini, 1980) and Kenya (Ruwa, 1984, 1990; Okemwa *et al.,* 1986). Tack (1992) and Tack *et al.* (1992) showed a negative correlation between shell length and height above chart datum. Oysters growing lower in the tidal area have a longer proportion of the day available for feeding, hence can be expected to grow faster. However, the increase in shell length is rather small compared with the increase of the time submerged. This rises the question whether the feeding behaviour of *S. cucullata* changes during the time the oyster stays submerged during each tidal cycle.

Several authors have studied the efficiency of particle retention from algal suspensions in bivalve molluscs (for reviews, see Jørgensen, 1960, 1975a; Winter, 1978; Bayne *et al.,* 1976a, b; Bayne and Newell, 1984). A majority of bivalve species retains particles ≥ 4 µm diameter with an efficiency of up to 100% while they retain particles of 1 µm diameter with a reduced efficiency of up to 50% (Haven and Morales-Alamo, 1970; Vahl, 1972 a, b, 1973 a, b; Jørgenson, 1975b; Møhlenberg and Riisgard, 1978; Palmer and Williams, 1980). There are, however, important and apparently adaptive differences between bivalves in their efficiency to retain fine particles. A clear illustration of those differences is found in a number of *Pecten* species. *Pecten opercularis* and *P. septemradiata* have ctenidia that lack eulaterofrontal ciliary tracts. They live on coarse deposits in open phytoplankton dominated waters. They show a decrease in retention efficiency of particles smaller than 7 µm At 1 µm their retention efficiency drops up to 20% (Møhlenberg and Riisgard, 1978). In this case the two *Pecten* species appear to be structurally adapted to exploit the larger suspended particles, including algal cells, as primary component of their diet.

The retention rate of large particles is clearly dependent on the ctenidium acting as a mechanical sieve. However Rubenstein and Koehl (1977), LaBarbera (1978), Jørgenson (1983), and Silvester and Sleigh (1984) present a good deal of evidence that the capture of fine particles not only depends on particle size, but also on electrical charge and other factors. Those factors allow the capture of fine particles including bacterioplankton with a much

greater efficiency than would be predicted from the mechanical properties of the ctenidial filter. There are a number of bivalves living near to bacteria rich detrital sources that are able to retain even bacterioplankton with a high efficiency. Wright *et al.* (1982) showed that the saltmarsh mussel *Geukensia demissa* was capable of the efficient clearance of natural bacterioplankton in contrast with *Mytilus edulis* and *Mya arenaria* which removed mainly the phytoplankton from mixed algal and bacterioplankton suspensions. Mwangi (1994) observed a reduction of bacterial biovolume in a mangrove creek due to *Saccostrea cucullata* feeding.

Berry and Schleyer (1983) showed that the mussel *Perna perna* has the capability to remove latex particles with a diameter of 0.46 µm from suspension. This diameter corresponds approximately with the diameter of the free living coccoid bacteria occurring in the water column over a reef off Durban, South Africa. Those bacteria are an important potential food source for the mussels. It appears that the ctenidium acts as a pre-ingestive site of particle selection in some bivalves. The spectrum of particles which can be retained appears to be an adaptation by the animals to exploit the local resource available in the water column.

A second potential site of pre-ingestive selection are the labial palps which sort material prior to ingestion. The oyster *Crassostrea virginica* is capable of food selection on a qualitative basis through rejection tracts on the labial palps (Loosanoff, 1949). *C. virginica*, fed with a mixed diet of silt (<32 µm diameter) and the alga *Tetraselmis suecica*, is able to reduce the concentration of the alga voided in the pseudofaeces by over 50% compared with levels in the food supply (Newell and Jordan, 1983). The authors suggested chemoselection as the main reason for the preferential ingestion of algae compared with inert salt particles. Peirson (1983) demonstrated differential utilisation of various algal species in the scallop *Argopecten irradians*. Also the deposit feeding bivalve *Macoma nasuta* rejects as much as 97% of the dry weight of material removed from the surface sediments. Only organic rich material is preferentially ingested by *M. nasuta*.

Shumway *et al.* (1985) made use of flow cytometry to estimate the clearance rate of six bivalve species, each in the presence of mixed cell suspensions of the dinoflagellate *Prorocentrum minimum*, the diatom *Phaeodactylum tricornutum*, and the cryptomonad flagellate *Chroomonas salina*. They also estimated the proportional occurrence in the pseudofaeces and faeces. *Ostrea edulis* preferentially clears the dinoflagellate compared with similar sized

cells of the diatom and the cryptomonad flagellate. The diatom was consistently and preferentially rejected in the pseudofaeces of *Ensis directus*, *Placopecten magellanicus* and *Arctica islandica*. In the majority of the bivalves from which they obtained faecal material, Shumway *et al.* (1985) found clear evidence of preferential absorption of the cryptomonad flagellate from the mixed diet which was ingested. The possibility of selective removal of particular components of the available food resource suggests that certain algae may be quantitatively more important in the diet of bivalves than their relative abundance under natural conditions might lead us to suppose.

Estuarine bivalve molluscs live and grow in the presence of substantial concentrations of suspended organic and inorganic material. These organisms have adapted to tolerate and even to benefit from their turbid environment (Urban and Kirchman, 1992). Barille *et al.* (1993) studied relative retention efficiency in *Crassostrea gigas*. They found the Japanese oyster has an active response to high particle concentrations. At low seston load (1.34 $mg.l^{-1}$), the oyster shows no change in retention for particles larger than 3-4 µm (Spheric Equivalent Diameter, SED). But at the highest concentration (64.37 $mg.l^{-1}$), it can only retain particles larger than 12 µm (SED) with maximum efficiencies.

Wright (1982) described the nutritional role of amino acids in filter-feeding marine invertebrates. Manahan (1983) studied the role of dissolved organic material in the nutrition of pelagic larvae. Manahan *et al.* (1982) demonstrated the net uptake of dissolved amino acids from natural seawater by the mussel *Mytilus edulis*. Van Evelghem (1996) showed an uptake of C^{14}-labelled glucose by *Saccostrea cucullata*. The highest concentrations of radioactive glucose were found in the gills. Tack and Polk (1996) found a significant difference in uptake of ink colloidals between several species of tropical bivalves. Coral reef species show a low uptake, while mangrove and seagrass meadow species show a high uptake rate. In the non-tropical bivalves from the North sea, a remarkable difference was observed between littoral and sublittoral species. Shallow-water forms exhibit higher uptake rates possibly due to higher DOC concentrations in surface waters.

The purpose of the present study was to determine whether selective uptake of particular and dissolved organic material occurs in *Saccostrea cucullata* (von Born, 1778). We also studied the possible changes in those processes in function of the time *S. cucullata* stays submerged.

Material and Methods

Collection and maintenance

Specimens of the mangrove oyster *Saccostrea cucullata* were collected at low water tidal level in Gazi Bay, a mangrove area adjacent to Gazi, a village 50 km south of Mombasa. All oysters collected were growing on *Rhizophora mucronata* trees within a rectangle of 8 by 2 meters. Oysters were collected in relation to their submersion time during each tidal cycle. Animals were transported to the laboratory where they were held in flow-through tanks in water pumped continuously from Tudor Creek. Tudor Creek is part of an extensive mangrove area around Mombasa. Because of the massive collection of *Saccostrea cucullata* in the mangrove areas around Mombasa we preferred to collect the oysters in Gazi Bay where naturally grown adult oysters are available.

Experimental procedure

For each food source (particulate organic carbon (POC), chlorophyl, phyto and zooplankton, bacteria, colloidal particles, dissolved organic carbon <0.2 µm or dissolved organic carbon between 0.2 and 1.2 µm) identical experiments were carried out. However, the last two food sources were sampled during the same series of experiments.
All experiments were carried out between 17th August 1993 and 31st August 1993 at the same time of day (between 8.00 a.m. and 8.00 p.m.). 24 hours prior to the first experiment, individual specimens were placed in Vitrost grazing chambers with a volume of 600 ml (see Fig. 1). Grazing chambers were placed in tanks with flowing seawater to maintain ambient temperature. Each of the grazing chambers can be subjected to a pre-programmed tidal cycle. The tidal cycle within the grazing chambers was running parallel with the real tidal cycle in Tudor Creek. This was necessary to simulate natural food availability for *Saccostrea cucullata*. The seawater in the grazing chambers was changed every 30 minutes during the first two hours taking care the oysters in the grazing chambers stayed submerged all the time. After the first two hours seawater in the grazing chambers was changed every hour. 12 series of 6 grazing chambers were programmed to simulate different heights

in the tidal cycle. In reality this means that 6 oysters stayed submerged for 30 minutes, 6 for 60 minutes, 6 for 90 minutes, 6 for 2 hours, 6 for 3 hours, 6 for 4 hours, 6 for 5 hours, 6 for 6 hours, 6 for 7 hours, 6 for 8 hours, 6 for 9 hours and 6 for 10 hours. Those oysters had similar submersion times under natural conditions. For each series of 6 grazing chambers 3 control grazing chambers were provided with an empty oyster shell to correct for changes in food concentrations unrelated to oyster activity. In the first four series of grazing chambers, experiments were performed during the last 30 minutes of the time submerged. In all other cases experiments were run during the last hour of the time submerged.
At the beginning and at the end of each experiment seawater samples were taken from the grazing chambers to determine the concentrations of one of the different food sources. Care was taken to prevent sampling of faeces and pseudofaeces particles at the end of the experiments, which would disturb uptake and clearance rate measurements (Hildreth, 1980).
The uptake and clearance rates of the different food sources were calculated from the differences in concentration between the initial and the end concentration of each food source using a control correction. Uptake and clearance rate are closely related with each other: clearance rate x initial concentration = uptake rate. For each submersion time the uptake and clearance rates of the above mentioned food sources were measured for 6 *S. cucullata* specimens.

Particulate Organic carbon (POC)

At the beginning and at the end of each 'POC experiment' a 600 ml seawater sample was taken. The samples were filtered on a Whatman GF/C filter with a diameter of 1.2 µm nominal pore size under low suction (<10 cm Hg vacuum). Filters were frozen at a temperature of -60 °C after packing them in aluminium foil. Filters were transported to our laboratory in Brussels for analysis.
The particulate organic carbon (POC) was measured by a Ströhlein Coulomat 702. This instrument performs an automatic coulometric titration. Carbon is transformed to carbon dioxide (CO_2), which is absorbed in an alkaline barium perchlorate ($Ba(ClO_4)_2$) solution. This causes a decrease of the pH of the solution. With barium hydroxide ($Ba(OH)_2$) formed by electrolysis, an automatic back titration to the initial pH takes place. A built in

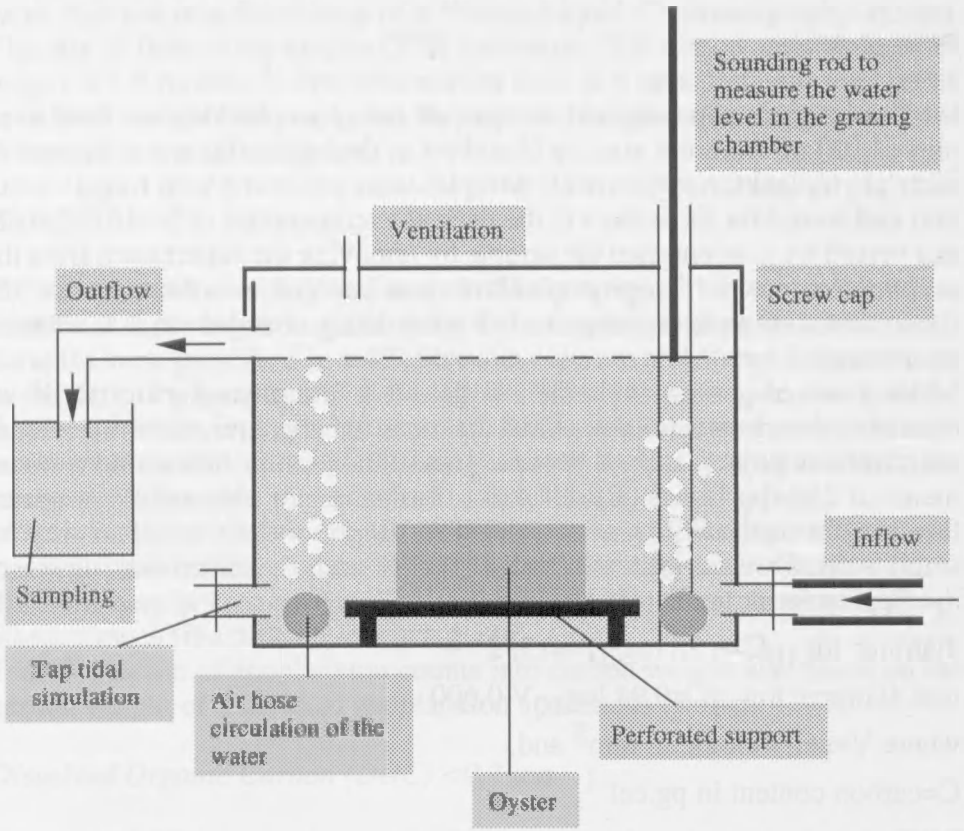

Figure 1. Diagram of a grazing chamber.

electronic unit transforms the measured amount of electricity used for the back titration into counts displayed, of which 1 count represents 2 x 10^{-7} g C. The POC is measured as the difference of the total particulate carbon (TPC) and the particulate inorganic carbon (PIC). To measure the TPC all the carbon present is oxidised to CO_2 in an oxygen stream while the filter is heated to 900 °C in a quartz tube. The PIC is measured by converting all the inorganic carbon present to CO_2 in an acid solution (8.5% orthophosphoric acid (ortho-H_3PO_4)).

Phytoplankton

For microscopic counting and analysis of the phytoplankton we took samples of 100 ml from the grazing chambers at the beginning and at the end of each 'phytoplankton experiment'. Samples were preserved with Lugol's solution and stored for three days in the dark at a temperature of 5 - 10 °C. After this period we concentrated the sample by removing the supernatant from the sedimented seston. The phytoplankton was counted in subsamples of the concentrated sample making use of a counting chamber and a reversed microscope.
Making use of geometric forms recognised in the phytoplankton cells we estimated the mean cell volume and the mean spheric equivalent diameter of the different phytoplankton genera. This estimation is based on measurements of 250 specimens of each genus. In the case of phytoplankton genera forming chains the SED was calculated on the mean chain length in stead of single cells. For each genus we calculated the carbon content making use of the Eppley formula (Eppley, 1978):

diatoms: $\log_{10}C=0.76 \log_{10}V-0.352$

non diatoms: $\log_{10}C=0.94 \log_{10}V-0.600$

where V=cell volume in mm^3 and,

C=carbon content in $pg.cel^{-1}$.

Chlorophyl

For the determination of chlorophyl concentrations 600 ml seawater was sampled at the beginning and at the end of each 'chlorophyl experiment'. The samples were filtered on a Whatman GF/C filter with a diameter of 1.2 µm nominal pore size under low suction (<10 cm Hg vacuum). Filters were frozen at a temperature of -60 °C after packing them in aluminium foil. Chlorophyl analyses were done at Brussels Free University.
Chlorophyl concentration in the samples was measured by high performance liquid chromatography (HPLC). We made use of a reversed phase C^{18} column. Chlorophyl identification and calibration was done on the basis of 12 chlorophyl standards between 0.05 and 10 $mg.m^{-3}$. The extracted samples

were injected in a fixed loop of a Waters Liquid Chromatography system. The rate of flow of the elution (75% methanol, 22% aceton and 3% distilled water) is 1.5 $ml.min^{-1}$. The total elution time is 8 min. Data are transferred to a DOS compatible computer. Results are calculated making use of the Waters baseline 810 software.

Zooplankton

For microscopic counting and analysis of the zooplankton we took samples of 600 ml at the beginning and at the end of each 'zooplankton experiment'. Samples were preserved in a 4% formalin solution and stored for three days in the dark at a temperature of 5 - 10 °C. After this period we concentrated the sample by removing the supernatant from the sedimented seston. At least one day before the counting of the zooplankton Bengal rose was added to the samples. All samples were counted completely (no subsampling).
Making use of geometric forms recognised in the zooplankton cells we estimated the mean cell volume and the mean spheric equivalent diameter of the different zooplankton genera. This estimation is based on measurements of 50 specimens (if available) of each genus.
Transformation of zooplankton counts into carbon weight was based on the carbon weight of individual zooplankton specimens (Bollen, 1993).

Dissolved Organic Carbon (DOC) <0.2 µm

Samples of 5 ml were taken at the beginning and at the end of each 'DOC experiment'. The samples were filtered through Millipore filters with 0.2 µm nominal pore size under low suction (<10 cm Hg vacuum). Samples were stored in small, glass-stoppered glass bottles with their mouths covered by aluminium foil. Samples were transported to Nairobi University at a temperature of -60° C. All samples were analysed within three days after sampling.
To 5 ml of filtered sample (in a 10 ml ampule) phosphoric acid and potassium persulphate were added. All inorganic carbonate was removed by passing nitrogen gas through the sample, and organic carbon in the water was oxidised to carbondioxide by heating the sealed ampule to 130 °C for 40 min. This carbondioxide was then estimated by dispelling it from the ampule in a stream of nitrogen gas that was passed through a Beckman non-disper-

sive infrared absorption gas analyser. A more comprehensive outline of the method is given by Strickland and Parsons (1972).

Dissolved Organic Carbon (DOC) between 0.2 µm and 1.2 µm

Dissolved organic carbon between 0.2 µm and 1.2 µm is calculated as the difference between dissolved organic carbon <1.2 µm and dissolved organic carbon <0.2 µm
Sampling and determination of DOC <1.2 µm was identical to that of DOC <0.2 µm However the filter used was a Whatman GF/C glass fibre filter with a nominal pore size of 1.2 µm Samples were taken within the same experiments determining DOC <0.2 µm

Colloidal melanin

To examine whether *S. cucullata* is able to consume colloidal DOC we offered suspensions of colloidal melanin from the ink sac of the cuttlefish *Sepia sp.* to each of the *S. cucullata* specimens (Tack and Polk, 1996). Colloidal melanin was chosen because of its high visibility, high stability (Bagnara and Hadley, 1973), and lack of tendency to agglutinate (Flood *et al.*, 1990).
During a complete tidal cycle we added colloidal melanin to the seawater flowing through the grazing chambers. *Sepia* ink, prefiltered through a Whatman GF/C glass fibre filter (1.2 µm nominal pore size), was added in a concentration of 10 $mg.l^{-1}$. This concentration approximates the naturally occurring dissolved organic carbon in a mangrove creek.
Removal of colloidal *Sepia* ink from the seawater by *S. cucullata* was measured as the change of absorbance of 500 nm light (using a spectrophotometer). Conversion to dry weight was done using dilution series made from a known mass of ink (Fig. 2).

Bacteria

100 ml samples were taken at the beginning and at the end of each 'bacteria experiment'. They were put into clean glass vials and preserved in 2% for-

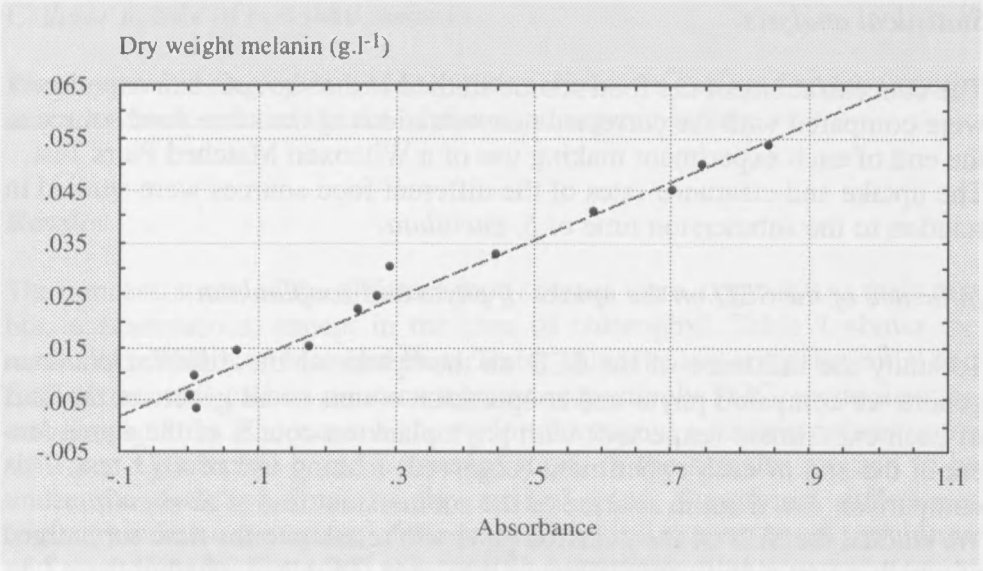

Figure 2. Linear correlation ($y=0.0074+0.0558x$, $r^2=0.972$, $n=13$, $p<10^{-4}$) between the absorbence of 500 nm light and the dry weight of melanin dissolved in one litre of seawater.

malin. Samples were stored at 4° C before analysis (within 4 months). To visualise the bacteria we made use of epifluorescence microscopy of acridine orange stained samples collected on 0.2 µm poresize polycarbonate filters (25 mm diameter). Counting and measurement was done at a total magnification of 1250 x under a Zeiss epifluorescence microscope fitted with a Plan-NEOFLUAR 100x/1.3 objective, a 10x ocular and an optivar of 1.25x. Counting and sizing of the bacteria was performed by an image analysis system using the Bact-Proza 3 software (NIOZ, Netherlands). Bacteria were counted in at least 10 randomly chosen counting fields (40 mm x 40 mm) with a minimum of 200 bacteria present. To convert bacteria biovolume to carbon weight a factor of 0.22 pg $C.mm^{-3}$ was used (Bratbak, 1985).

Statistical analysis

The concentrations of the food source studied at the start of each experiment were compared with the corrected concentrations of the same food source at the end of each experiment making use of a Wilcoxon Matched Pairs Test. The uptake and clearance rates of the different food sources were studied in relation to the submersion time of *S. cucullata*.

Influence of the SED on the uptake of phyto and zooplankton

To study the influence of the SED on the uptake of the different plankton genera we compared phyto and zooplankton counts of all genera at the start of each experiment (expected) with phytoplankton counts of the same genera at the end of each experiment (observed) making use of a χ^2 test. This comparison was done in relation to the submersion time of *S. cucullata*.
We studied the SED of the plankton genera in relation to the time submerged at which a first significant difference was seen. To detect whether there was a significant relation between both variables we used Spearman rank order correlation and Kendall tau correlation coefficients.

$\delta^{13}C$ measurements

At the end of the feeding experiments three oysters of each time series were used to determine $\delta^{13}C$ values. Those values were compared with the $\delta^{13}C$ values of possible food sources. 20% HCl was used to remove carbonates present. Dry animal tissue was ground to a fine powder using a mortar, pestle and liquid nitrogen.
Mass spectrometric measurements were performed using a Delta E Finnigan Mat isotope ratio mass spectrophotometer. CO_2 generated in the CN analyser was automatically trapped in an on-line CT-NT Finnigan Mat Trapping Box for cryopurification before being injected into the Mass Spectrophotometer. A graphite reference material (USG-24) was used as a standard for carbon isotopic measurement. Values are expressed relative to the Vienna Peedee Belemnite standard. Stable carbon isotope abundances are presented as $\delta^{13}C$ values.

Cellular uptake of colloidal melanin

The three remaining oysters of each time series were analysed under a microscope to check cellular uptake of dissolved organic carbon.

Results

The concentrations of the different food sources were expressed as their carbon concentrations, except in the case of chlorophyl. Table 1 shows the results of a Wilcoxon Matched Pairs Test (t values) comparing the initial and final (corrected) carbon concentration of respectively POC, phytoplankton and zooplankton. The same is shown for chlorophyl a concentrations in the seawater. Chlorophyl b and c concentrations in the samples seemed to be under the detection limit of the method used. Significant differences ($p<0.01$) indicate the uptake of the respective food sources by all specimens of *S. cucullata* (n=6) studied at a specific submersion time.

No significant differences in POC concentrations are measured at submersion times of 30, 60, 90 and 120 min. A first significant difference between the initial and final POC concentrations is seen between the second and the third hour after the oysters are submerged. Significant differences are measured until the maximum submersion time of 10 hours. Phytoplankton concentrations show significant differences from the first moment the oyster is submerged until the maximum submersion time. Chlorophyl a and zooplankton concentrations show similar results as the total POC concentrations.

Table 2 shows the t values of a Wilcoxon Matched Pair Test comparing the different DOC concentrations at the beginning and at the end of each experiment. DOC concentrations for DOC <0.2 µm show no significant differences while the DOC concentrations of DOC in the size range of 0.2 to 1.2 µm show significant differences from the first moment *S. cucullata* is submerged. The initial concentrations of colloidal melanin and bacteria are both significant different from the concentrations of colloidal melanin and bacteria at the end of the experiments up to respectively the fourth and the sixth hour if they stay submerged that long.

Comparing the concentrations of the different food sources at the beginning and at the end of each control experiment making use of a Wilcoxon

Time (h)	POC	Phytoplankton	Chlorophyll A	Zooplankton
0.5	4.0	0.0*	7.0	5.0
1	7.0	0.0*	9.0	1.0
1.5	9.5	0.0*	9.0	10.0
2	1.0	0.0*	7.0	2.0
3	0.0*	0.0*	1.0	0.0*
4	0.0*	0.0*	0.0*	0.0*
5	0.0*	0.0*	0.0*	0.0*
6	0.0*	0.0*	1.0	0.0*
7	0.0*	0.0*	0.0*	0.0*
8	0.0*	0.0*	0.0*	0.0*
9	0.0*	0.0*	0.0*	0.0*
10	0.0*	0.0*	0.0*	0.0*

Table 1. Wilcoxon Matched Pairs Test (t values) comparing the carbon concentration of respectively POC, phytoplankton and zooplankton in 600 ml seawater at the beginning and at the end of each experiment. The same comparison is made for the chlorophyll a concentration. Significant differences are marked with * ($p<0.01$).

Time (h)	DOC (<0.2µm)	DOC (>O.2µm and <1.2µm)	Colloidal melanin (>O.2µm and <1.2µm)	Bacteria
0.5	4.0	0.0*	0.0*	0.0*
1	6.0	0.0*	0.0*	0.0*
1.5	9.0	0.0*	0.0*	0.0*
2	5.0	0.0*	2.0	0.0*
3	7.0	0.0*	0.0*	0.0*
4	9.0	0.0*	0.0*	0.0*
5	6.0	0.0*	5.0	0.0*
6	6.0	0.0*	4.5	0.0*
7	9.0	0.0*	4.5	10.5
8	1.0	0.0*	4.0	7.0
9	10.0	0.0*	9.5	6.5
10	0.0*	0.0*	8.0	4.5

Table 2. Wilcoxon Matched Pairs Test (t values) comparing the carbon concentration of respectively DOC <0.2 µm, DOC with a size range between 0.2 and 1.2 µm, colloidal melanin and bacteria in 600 ml seawater at the beginning and at the end of each experiment. Significant differences are marked with * ($p<0.01$).

Matched Pairs Test shows no significant differences at all.
The median, minimum and maximum uptake rate of 6 *S. cucullata* specimens, measured in relation to their submersion time, is given in Table 3a for POC and phytoplankton, in Table 3b for chlorophyll a and zooplankton, in Table 4a for DOC <0.2 µm and DOC between 0.2 µm and 1.2 µm and in Table 4b for colloidal melanin between 0.2 and 1.2 µm and for bacteria. Tables 3 and 4 also give the percentages of the initial amounts of each food source, available in the grazing chambers, taken up by *S. cucullata* during the last hour of its submersion time.
The uptake and clearance rates of *S. cucullata* for POC as a function of the submersion time of the oyster are shown in Figure 3a and b. The uptake and clearance rates of *S. cucullata* for POC during the first 2 hours are either non-existent or relatively small compared with the uptake and clearance rates of *S. cucullata* for POC after the second hour. Only between the second and the third hour the uptake and clearance rates of *S. cucullata* for POC are measurable. Between the seventh and the tenth hour the uptake and clearance rates of *S. cucullata* for POC increase rapidly, reaching a maximum at the end of the submersion time. Figure 4a and b show negative uptake and clearance rates of *S. cucullata* for phytoplankton during the first 90 minutes *S. cucullata* is submerged. After a submersion time of 90 minutes the uptake rate increases fast to 4.10^{-4} mg $C.h^{-1}.ind^{-1}$, while the clearance rate increases from ±0 to 180 $ml.l^{-1}.ind^{-1}$. Between the third and the tenth hour the uptake rate of *S. cucullata* for phytoplankton remains more or less stable between 4.10^{-4} and 6.10^{-4} mg $C.h^{-1}.ind^{-1}$. This stability is even clearer when we have a look at the clearance rate (Fig. 4b). Uptake and clearance rates for chlorophyll a (Fig. 5a and b) are showing similar patterns as the uptake and clearance rates for phytoplankton. Figure 6a and b show the uptake and clearance rates of *S. cucullata* for zooplankton. The uptake and clearance rates are either non-existent or relatively small during the first two hours *S. cucullata* is submerged. There is a very fast increase in the uptake and clearance rates of *S. cucullata* for zooplankton between the second and the third hour after submersion. After the third hour the increase decreases.
The uptake and clearance rates of *S. cucullata* for DOC <0.2 µm were not significant different from zero. The uptake and clearance rates of *S. cucullata* for respectively DOC between 0.2 and 1.2 µm (Fig. 7a and b), colloidal melanin (Fig. 8a and b) and bacteria (Fig. 9a and b) show a completely different picture compared with the uptake rate of *S. cucullata* for POC and its

Time (h)	Food source: POC — Uptake rate (mg C.h^{-1}.ind^{-1}) Med.	Min.	Max.	POC — Percent of available POC (%) Med.	Min.	Max.	Phytoplankton — Uptake rate (x 10^{-3}) (mg C.h^{-1}.ind^{-1}) Med.	Min.	Max.	Phytoplankton — Percent of available phytoplankton (%) Med.	Min.	Max.
0.5	0.000	-0.003	0.003	0.1	-0.5	0.4	-0.05	-0.14	-0.02	-1.3	-3.4	-0.5
1	0.000	-0.002	0.001	-0.1	-0.4	0.2	-0.09	-0.14	-0.02	-1.3	-3.4	-0.5
1.5	0.000	-0.002	0.002	-0.1	-0.4	0.5	-0.11	-0.13	-0.07	-3.3	-4.2	-2.1
2	0.001	0.000	0.002	0.2	0.0	0.4	0.13	0.08	0.25	4.4	2.7	8.1
3	0.004	0.003	0.007	1.6	1.1	3.5	0.36	0.33	0.39	28.1	27.1	29.9
4	0.021	0.014	0.029	9.5	6.7	15.0	0.42	0.38	0.46	30.4	28.5	33.1
5	0.015	0.007	0.022	6.3	3.2	7.3	0.44	0.41	0.46	30.7	28.1	33.5
6	0.020	0.013	0.023	8.0	5.3	9.3	0.45	0.43	0.49	29.8	26.9	31.8
7	0.012	0.005	0.020	4.9	2.6	8.5	0.46	0.41	0.51	30.0	27.9	33.1
8	0.086	0.041	0.093	24.0	13.2	25.0	0.45	0.41	0.47	29.2	27.6	31.4
9	0.080	0.037	0.111	27.1	14.7	37.7	0.56	0.45	0.61	30.7	26.3	32.7
10	0.121	0.055	0.140	47.3	29.2	54.0	56.0	0.48	0.66	30.4	28.7	36.3

Table 3a. Saccostrea cucullata: median, minimum and maximum uptake rates of POC and phytoplankton as a function of the submersion time. Also shown are the median, minimum and maximum percentages of the initial amounts of POC and phytoplankton (available in the grazing chamber at the beginning of the experiment) taken up by *S. cucullata* during the last hour of its submersion time.

Time (min)	Food source: Chlorophyll a — Uptake rate (10^{-3} mg C.h^{-1}.ind^{-1}) Med.	Min.	Max.	Chlorophyll a — Percent of available chlorophyll a (%) Med.	Min.	Max.	Zooplankton — Uptake rate (mg C.h^{-1}.ind^{-1}) Med.	Min.	Max.	Zooplankton — Percent of available zooplankton (%) Med.	Min.	Max.
30	0.03	-0.18	0.13	1.9	-14.4	8.3	0.005	-0.006	0.008	2.0	-2.3	3.2
60	0.02	-0.19	0.16	1.2	-19.6	13.0	0.000	-0.004	0.006	0.0	-1.5	2.2
90	0.01	-0.09	0.11	0.5	-8.5	10.9	0.000	-0.008	0.008	0.0	-2.7	2.9
120	0.05	-0.08	0.13	4.4	-6.9	11.2	0.002	-0.004	0.010	0.7	-1.5	4.1
180	0.12	-0.02	0.16	15.1	-3.3	22.5	0.032	0.026	0.043	22.7	17.2	24.9
240	0.12	0.02	0.18	15.0	3.2	23.1	0.032	0.029	0.036	22.4	20.1	27.3
300	0.10	0.02	0.20	10.6	2.1	24.1	0.038	0.030	0.042	27.5	25.6	32.3
360	0.10	-0.02	0.16	12.6	-3.2	21.4	0.042	0.037	0.045	35.5	33.6	37.2
420	0.09	0.06	0.18	12.6	7.0	21.0	0.046	0.039	0.047	37.0	34.2	39.7
480	0.08	0.00	0.15	9.0	0.0	17.5	0.041	0.037	0.043	35.8	34.9	39.8
540	0.07	0.01	0.20	10.0	1.3	24.6	0.039	0.032	0.042	36.9	30.8	38.0
600	0.08	0.01	0.16	14.0	0.9	22.9	0.037	0.036	0.042	35.0	34.7	36.9

Table 3b. Saccostrea cucullata: median, minimum and maximum uptake rates of chlorophyll a and zooplankton as a function of the submersion time. Also shown are the median, minimum and maximum percentages of the initial amounts of chlorophyll a and zooplankton (available in the grazing chamber at the beginning of the experiment) taken up by *S. cucullata* during the last hour of its submersion time.

	Food source											
	DOC (< 0.2 µm						DOC (> 0.2 µm and < 1.2 µm)					
	Uptake rate ($mg\ C.h^{-1}.ind^{-1}$)			Percent of available DOC (< 0.2 µm) (%)			Uptake rate ($mg\ C.h^{-1}.ind^{-1}$)			Percent of available DOC (> 0.2 µm and < 1.2 µm) (%)		
Time (h)	Med.	Min.	Max.	Med.	Min.	Max.	Med.	Min.	Max.	Med.	Min.	Max.
0.5	-0.30	-0.43	0.28	-3.4	-5.4	4.0	4.11	3.31	4.38	19.8	18.9	25.5
1	-0.17	-0.59	0.26	-2.0	-6.9	2.8	1.13	0.63	1.55	6.1	3.7	10.1
1.5	-0.04	-0.55	0.35	-0.4	-5.9	4.0	1.05	0.82	1.39	8.7	5.3	10.2
2	0.10	-0.22	0.51	1.2	-2.8	5.6	0.40	0.03	0.45	2.6	0.2	3.4
3	-0.07	-0.57	0.15	-2.4	-12.8	3.6	0.56	0.41	0.78	6.8	4.1	14.3
4	-0.05	-0.35	0.49	-1.5	-10.6	10.7	0.39	0.25	0.44	3.7	2.3	5.4
5	-0.14	-0.48	0.48	-3.9	-12.2	13.5	0.20	0.13	0.29	2.7	1.5	4.8
6	0.16	-0.25	0.43	4.4	-6.6	9.4	0.17	0.04	0.23	1.6	0.4	3.0
7	-0.05	-0.44	0.33	-1.1	-9.8	7.8	0.18	0.06	0.25	2.0	0.6	3.4
8	0.12	-0.07	0.51	2.9	-2.0	14.9	0.16	0.09	0.23	1.4	0.9	3.1
9	0.04	-0.51	0.40	0.9	-12.9	9.4	0.18	0.14	0.24	2.0	1.3	3.2
10	-0.39	-0.66	-0.02	-10.3	-14.8	-0.6	0.14	0.11	0.26	1.8	1.0	2.9

Table 4a. Saccostrea cucullata: median, minimum and maximum uptake rates of DOC <0.2 µm and DOC in the size range 0.2 - 1.2 µm as a function of the submersion time. Also shown are the median, minimum and maximum percentages of the initial amounts of DOC <0.2 µm and DOC in the size range 0.2 - 1.2 µm (available in the grazing chamber at the beginning of the experiment) taken up by *S. cucullata* during the last hour of its submersion time.

Time (min)	Food source: Colloidal melanin (> 0.2 µm and < 1.2 µm) — Uptake rate (mg C.h^{-1}.ind^{-1}) Med.	Min.	Max.	Percent of available colloidal melanin (%) Med.	Min.	Max.	Food source: Bacteria — Uptake rate (mg C.h^{-1}.ind^{-1}) Med.	Min.	Max.	Percent of available bacteria (%) Med.	Min.	Max.
0.5	1.80	1.40	1.96	9.0	7.0	9.8	0.30	0.28	0.32	82.8	75.9	93.2
1	0.45	0.17	0.60	2.2	0.9	3.0	0.09	0.08	0.10	22.7	20.6	25.7
1.5	0.42	0.33	0.58	2.1	1.6	2.9	0.04	0.03	0.06	11.6	7.9	17.0
2	0.09	-0.07	0.15	0.46	-0.37	0.73	0.04	0.02	0.05	9.5	6.4	14.1
3	0.18	0.13	0.27	1.8	1.3	2.7	0.02	0.01	0.02	9.4	4.3	12.6
4	0.09	0.06	0.12	0.9	0.6	1.2	0.01	0.01	0.01	6.5	5.1	8.4
5	0.01	-0.02	0.04	0.1	-0.2	0.4	0.00	0.00	0.00	1.7	0.0	3.3
6	0.00	-0.05	0.01	0.0	-0.5	0.9	0.00	0.00	0.00	0.0	-1.8	2.6
7	-0.01	-0.02	0.01	-0.1	-0.3	0.1	0.00	0.00	0.00	0.9	-2.4	2.5
8	0.02	-0.02	0.03	0.2	-0.2	0.3	0.00	0.00	0.00	-0.6	-3.3	1.7
9	0.00	-0.04	0.04	0.0	-0.4	0.4	0.00	-0.01	0.01	-0.5	-4.1	5.4
10	0.00	-0.02	0.02	0.0	-0.2	0.2	0.00	-0.01	0.01	-1.9	-4.2	2.5

Table 4b. Saccostrea cucullata: median, minimum and maximum uptake rates of colloidal melanin and bacteria as a function of the submersion time. Also shown are the median, minimum and maximum percentages of the initial amounts of colloidal melanin and bacteria (available in the grazing chamber at the beginning of the experiment) taken up by *S. cucullata* during the last hour of its submersion time.

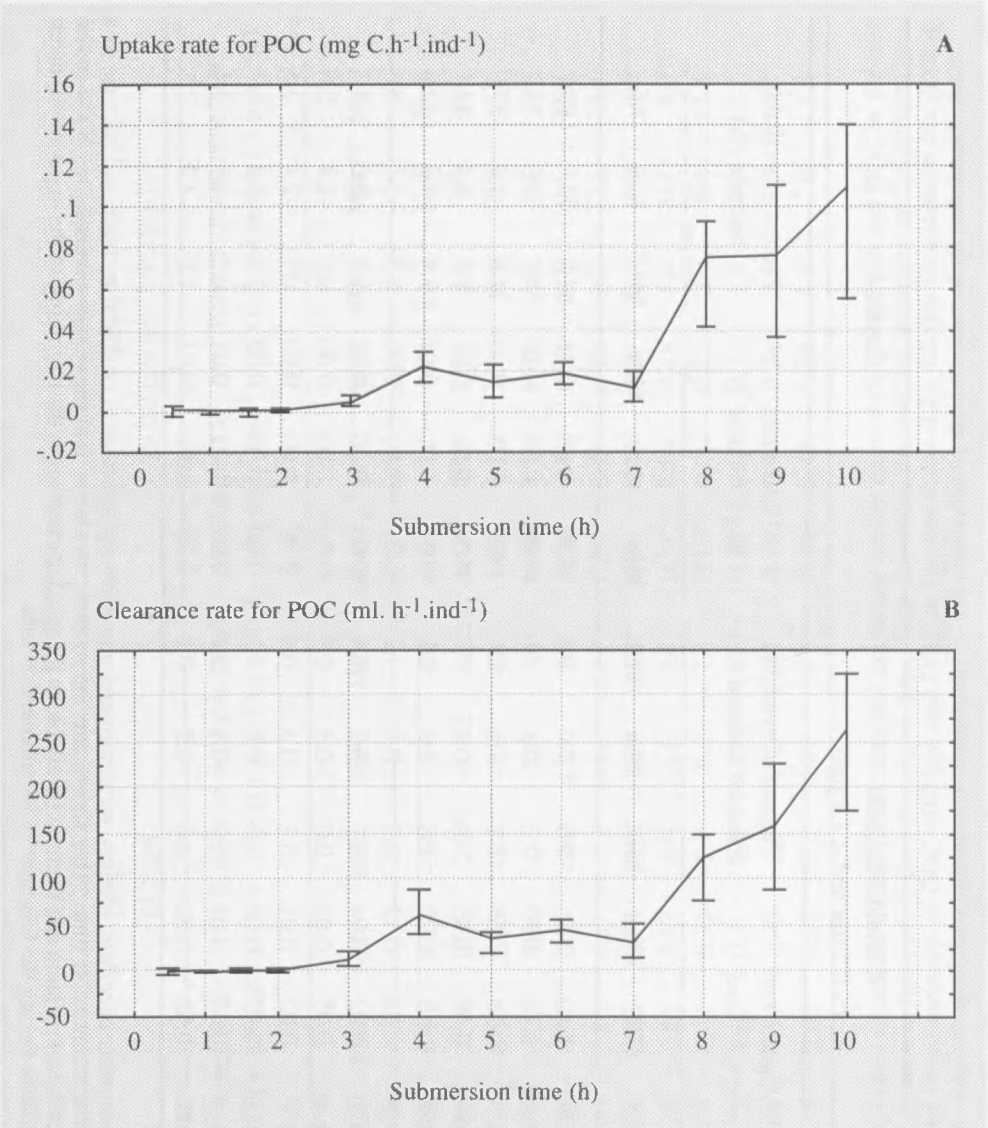

Figure 3. A: uptake rate of *S. cucullata* for Particulate Organic Carbon (POC) (mg $C.h^{-1}.ind^{-1}$) in function of its submersion time (h). B: clearance rate of *S. cucullata* for Particulate Organic Carbon (POC) ($ml.h^{-1}.ind^{-1}$) in function of its submersion time (h).

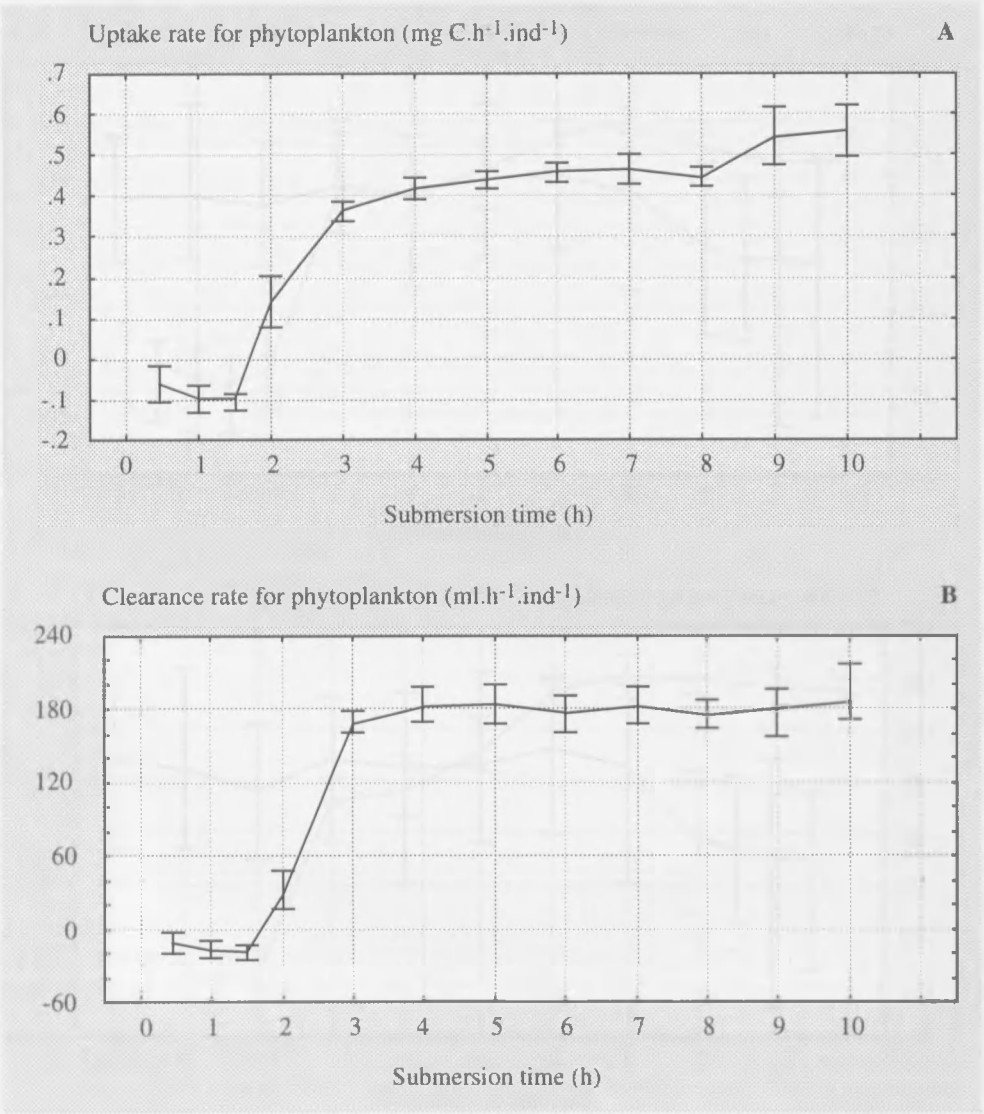

Figure 4. A: uptake rate of *S. cucullata* for phytoplankton (mg $C.h^{-1}.ind^{-1}$) in function of its submersion time (h). B: clearance rate of *S. cucullata* for phytoplankton ($ml.h^{-1}.ind^{-1}$) in function of its submersion time (h).

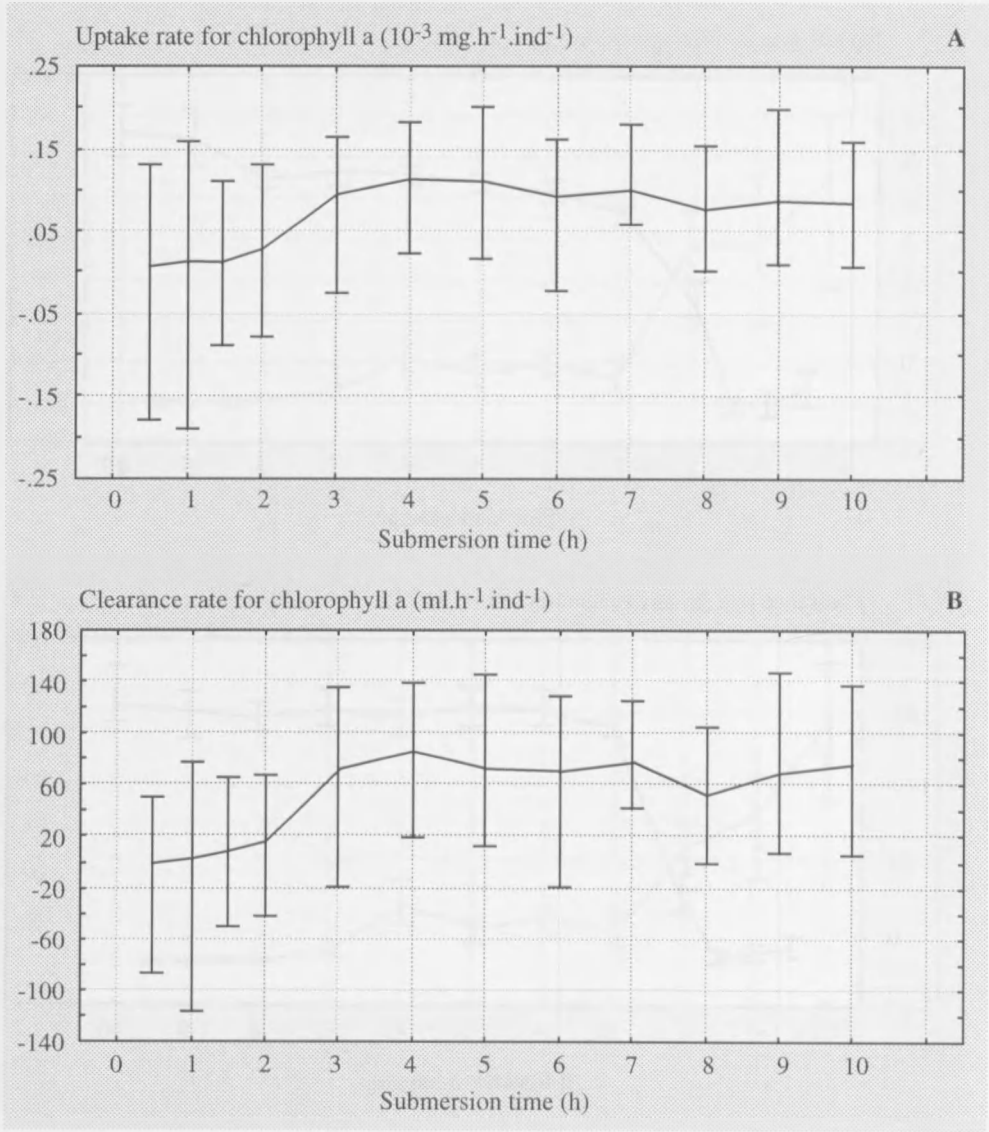

Figure 5. A: uptake rate of *S. cucullata* for chlorophyll a (mg.h^{-1}.ind^{-1}) in function of its submersion time (h). B: clearance rate of *S. cucullata* for chlorophyll a (ml.h^{-1}.ind^{-1}) in function of its submersion time (h).

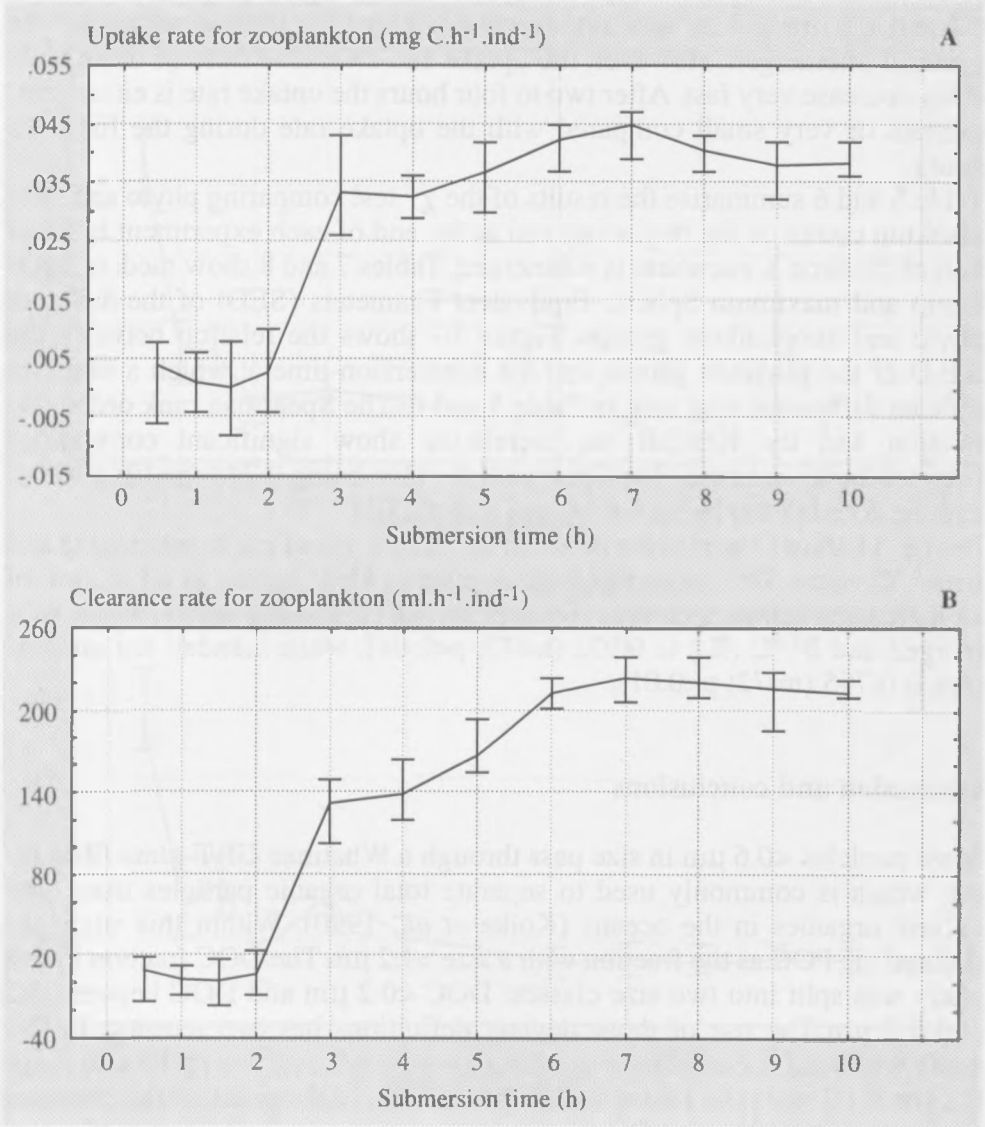

Figure 6. A: uptake rate of *S. cucullata* for zooplankton (mg $C.h^{-1}.ind^{-1}$) in function of its submersion time (h). B: clearance rate of *S. cucullata* for zooplankton ($ml.h^{-1}.ind^{-1}$) in function of its submersion time (h).

components. The uptake rates of *S. cucullata* for respectively DOC between 0.2 and 1.2 µm and its subcomponents are very high immediately after the oyster is submerged. However, the uptake and clearance rates of those variables decrease very fast. After two to four hours the uptake rate is either non-existent or very small compared with the uptake rate during the first two hours.

Table 5 and 6 summarise the results of the χ^2 test comparing phyto and zooplankton counts at the beginning and at the end of each experiment in function of the time *S. cucullata* is submerged. Tables 7 and 8 show median, minimum and maximum Spheric Equivalent Diameters (SED) of the different phyto and zooplankton groups. Figure 10 shows the relation between the S.E.D of the plankton genera and the submersion time at which a first significant difference was seen in Table 5 and 6. The Spearman rank order correlation and the Kendall tau correlation show significant correlations between both variables. The Spearman R value being 0.744 ($n=12$; $p<0.01$) and the Kendall tau being 0.614 ($n=12$; $p<0.001$).

Figure 11 shows the relation between the time *S. cucullata* is submerged and its $\delta^{13}C$ value. We can see a clear increase in $\delta^{13}C$ values as a function of an increasing submersion time. Spearman rank correlation between time submerged and $\delta^{13}C$ (‰) is 0.932 ($n=72$; $p<0.01$), while Kendall tau correlation is 0.795 ($n=72$; $p<0.01$).

Discussion and conclusions

Most particles <0.6 µm in size pass through a Whatman GF/F glass fibre filter, which is commonly used to separate total organic particles from 'dissolved' organics in the oceans (Koike *et al.*, 1990). Within this study we defined the POC as the fraction with a size >1.2 µm The DOC fraction in this study was split into two size classes: DOC <0.2 µm and DOC between 0.2 and 1.2 µm The use of those deviant definitions has two reasons. In this study we found *S. cucullata* was taking up especially carbon in the size range 0.2 µm - 1.2 µm (see Tables 3a, 3b, 4a and 4b). Making use of the common separation of DOC and POC the uptake and clearance rates within this size range would be less clear. Secondly, DOC in the size range 0.2 - 1.2 µm exists especially of bacteria and colloidal material. The bacteria had a minimum diameter of 0.314 µm and a maximum diameter of 0.396 µm They

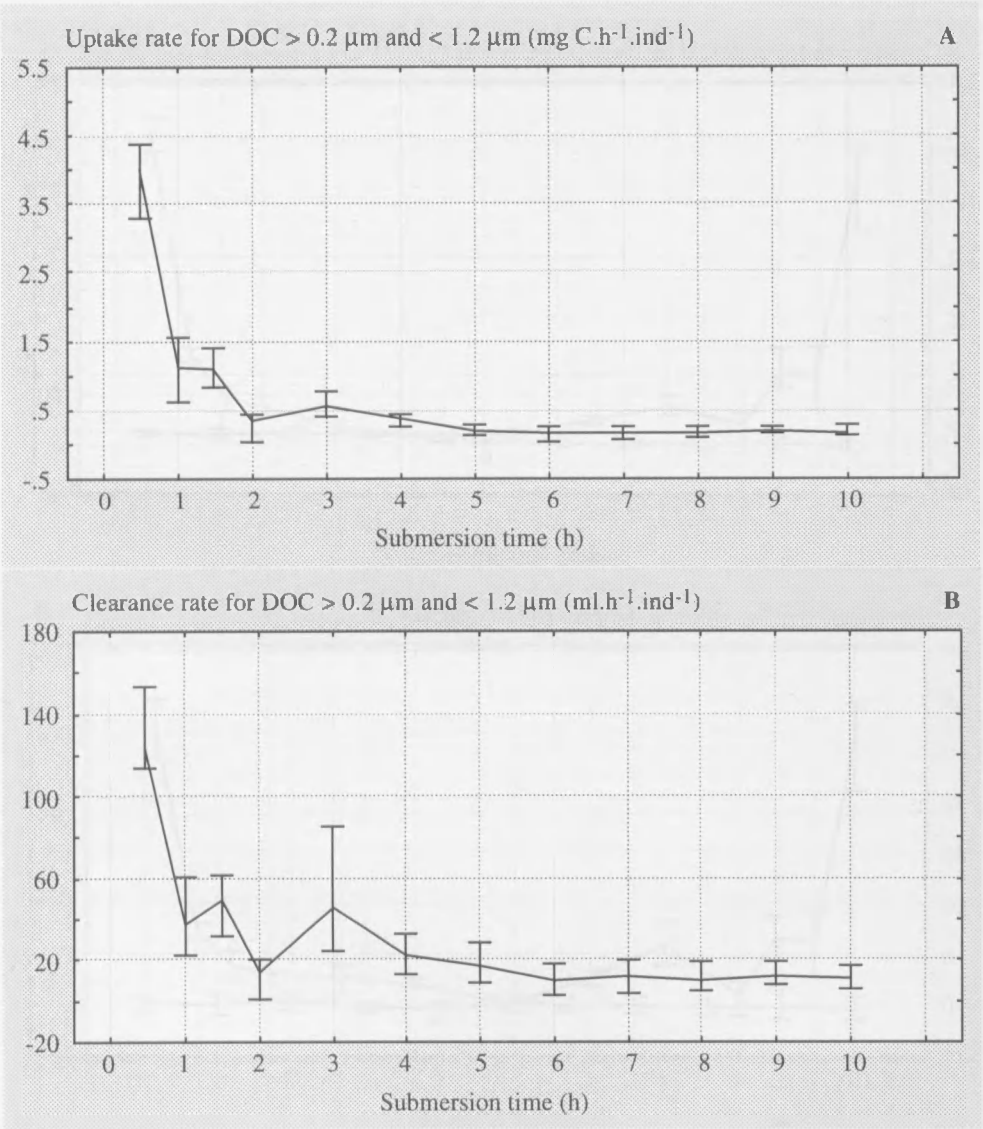

Figure 7. A: uptake rate of *S. cucullata* for Dissolved Organic Carbon (DOC) between 0.2 and 1.2 µm (mg $C.h^{-1}.ind^{-1}$) in function of its submersion time (h). B: clearance rate of *S. cucullata* for Dissolved Organic Carbon (DOC) between 0.2 and 1.2 µm ($ml.h^{-1}.ind^{-1}$) in function of its submersion time (h).

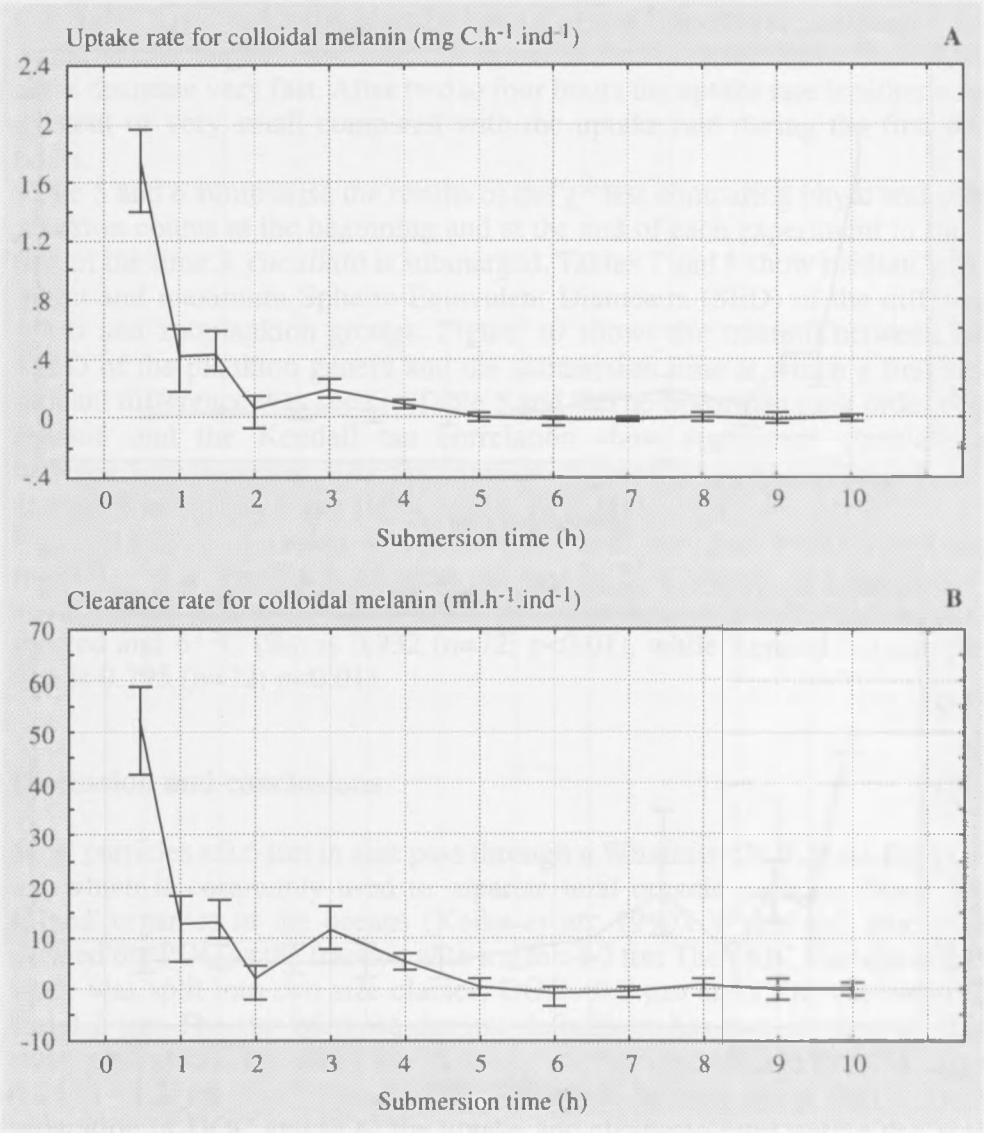

Figure 8. A: uptake rate of *S. cucullata* for colloidal melanin (mg $C.h^{-1}.ind^{-1}$) in function of its submersion time (h). B: clearance rate of *S. cucullata* for colloidal melanin ($ml.h^{-1}.ind^{-1}$) in function of its submersion time (h).

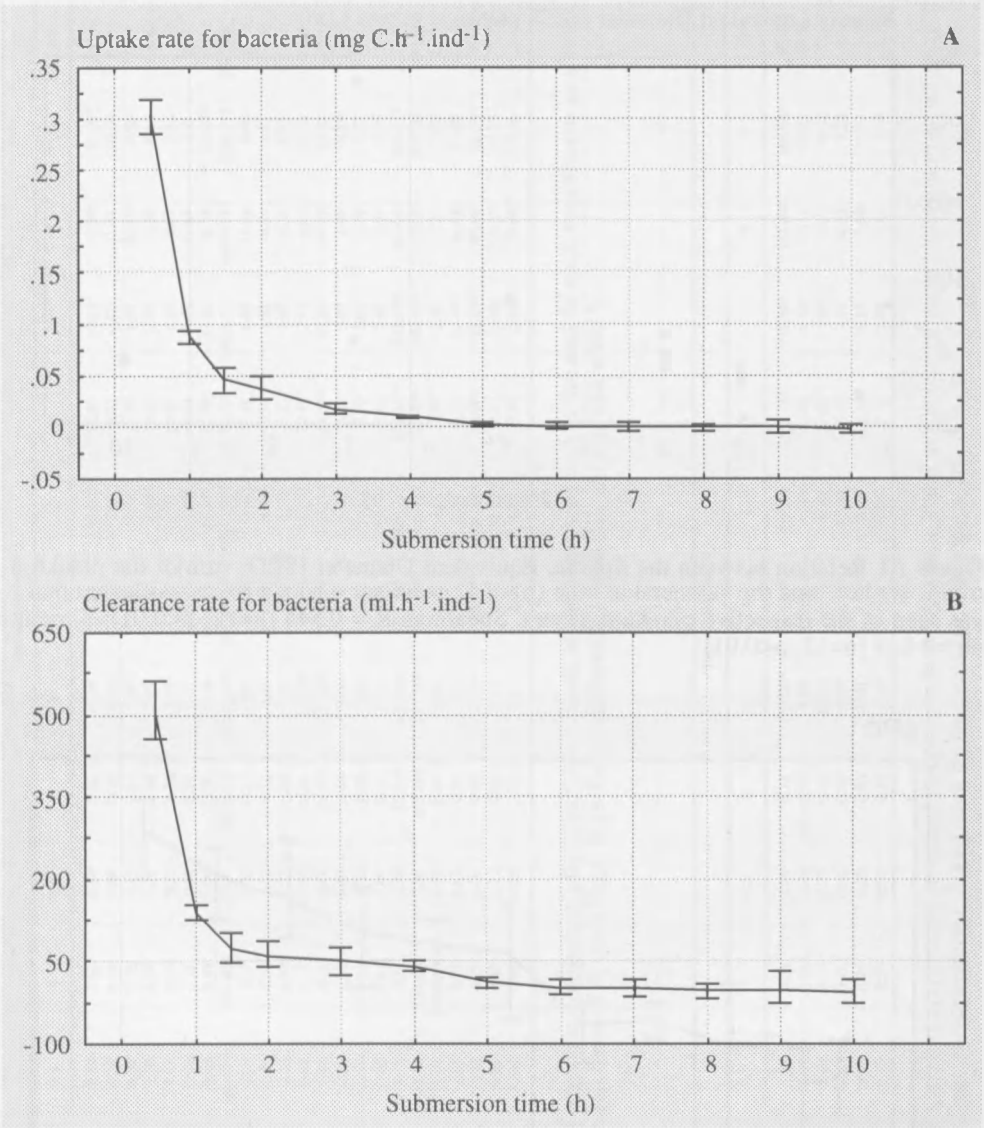

Figure 9. A: uptake rate of *S. cucullata* for bacteria (mg $C.h^{-1}.ind^{-1}$) in function of its submersion time (h). B: clearance rate of *S. cucullata* for bacteria ($ml.h^{-1}.ind^{-1}$) in function of its submersion time (h).

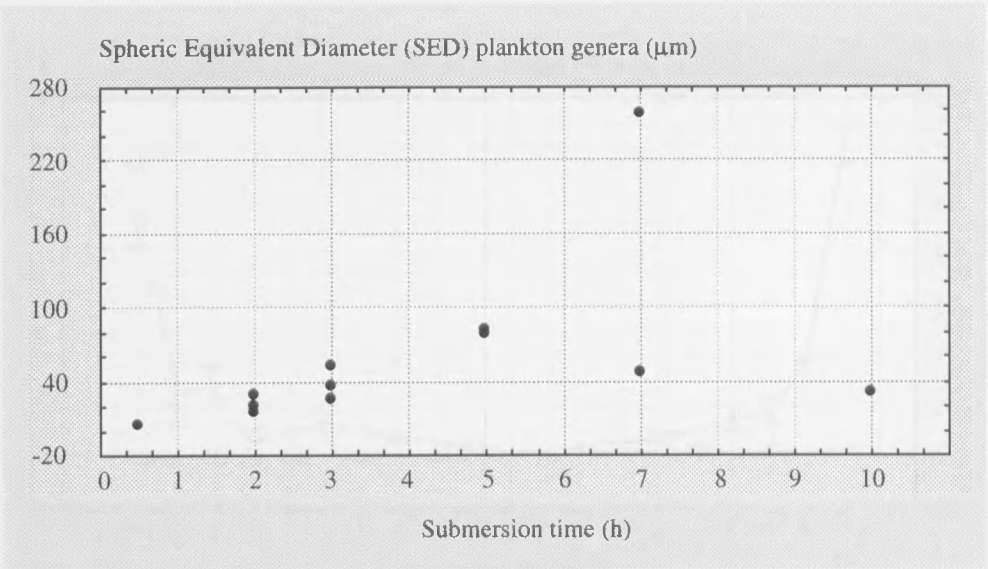

Figure 10. Relation between the Spheric Equivalent Diameter (SED) (µm)of the plankton groups studied and the submersion time (h) of *S. cucullata* where a first significant uptake was seen of the respective plankton genera. Spearman R = 0.744 (n=12; p<0.01); Kendall tau =0.614 (n=12; p<0.01).

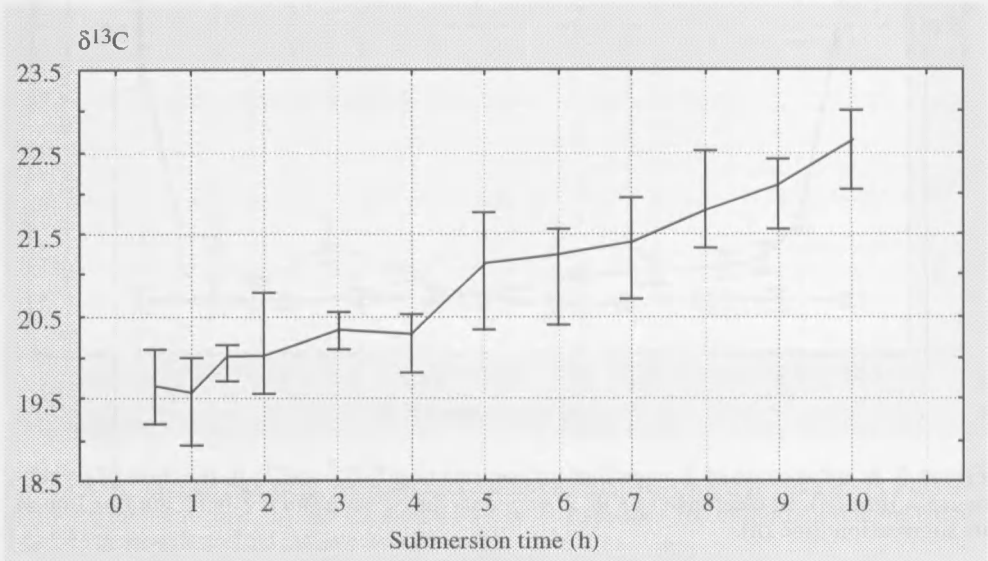

Figure 11. Relation between the $\delta^{13}C$ (‰) of *S. cucullata* and its submersion time (h). Spearman R=0.932 (n=72; p<0.01); Kendall tau =0.795 (n=72; p<0.01).

Genera	Time submersed (h)											
	0.5	1	1.5	2	3	4	5	6	7	8	9	10
Actinoptychus	0.59	0.32	0.84	0.66	8.34	10.73	15.62*	12.52	19.44*	17.20*	17.59*	25.14*
Amphipmra	0.29	0.54	1.08	0.19	4.23	4.86	5.04	9.02	8.08	11.44	10.54	10.08
Amphora	0.30	0.52	0.55	2.83	7.30	8.35	11.24	9.59	9.99	12.52	8.93	15.80*
Ast.+Mer.+Sur.	8.10	8.19	7.66	2.48	1.27	2.08	0.81	0.66	0.34	0.20	0.14	0.46
Lic.+Rhi.+Aur.+Cli.	3.71	5.80	5.39	9.76	1.57	0.58	0.81	0.93	3.32	3.47	2.23	1.45
Bacillaria	0.74	1.11	0.98	3.28	1.37	5.08	6.78	5.83	8.93	11.87	7.21	11.41
Biddulphia	0.49	0.59	1.06	0.99	8.58	10.85	15.89*	16.36*	12.66	6.38	20.84*	14.57
Chaetoceros	669.38*	993.09*	170.8*	29679.13*	21308.49*	22838.06*	20580.53*	17271.39*	16956.55*	10999.77*	23224.17*	29405.55*
Coscinodiscus	0.35	1.17	0.35	0.21	8.57	7.68	9.47	11.11	8.53	12.06	8.60	11.99
Cyclotella	1.66	1.34	1.12	1.12	1.42	1.98	4.31	4.62	3.53	4.24	3.97	4.83
Diploneis	1.19	0.87	1.33	0.84	4.72	1.64	1.77	2.87	1.65	0.35	0.65	1.69
Grammatophora	0.92	1.76	1.31	0.26	15.18*	24.70*	19.17*	18.43*	12.17	9.92	12.62	15.73*
Gyrodinium	1.74	4.21	2.44	119.15*	137.28*	174.27*	126.53*	68.88*	43.58*	50.29*	74.85*	70.33*
Gyrosigma	0.52	0.51	0.67	0.46	8.44	7.36	5.48	4.27	6.39	4.45	8.79	5.70
Lymbia	1.37	1.21	1.32	0.88	2.30	2.48	1.12	2.32	2.04	2.43	1.32	1.77
Melosira	0.43	0.81	1.04	0.09	0.15	0.10	1.17	0.61	3.59	3.51	5.95	9.05
Navicula	6.78	3.14	3.34	49.93*	203.24*	212.38*	220.21*	228.83*	243.59*	307.01*	248.71*	207.39*
Nitzschia	10.50	10.62	7.36	313.10*	374.18*	272.18*	330.15*	339.58*	408.98*	433.62*	347.39*	437.39
Nitzschiella	1.03	0.45	0.55	0.19	4.80	6.43	6.09	3.99	3.90	5.28	5.95	10.86
Oscillatoria	1.05	0.73	0.63	0.28	7.81	10.11	8.05	8.24	16.44*	16.68*	13.55	12.46
Peridinium	2.72	4.71	2.09	9.44	26.58*	32.02*	46.87*	33.07*	45.10*	48.66*	53.93*	30.36*
Pleurosigma	0.51	0.43	1.22	0.69	3.11	1.82	1.22	2.27	0.80	0.37	3.53	6.77
Rhabdonema	1.42	0.98	0.51	1.07	2.13	2.48	4.18	8.45	6.07	8.08	7.36	6.84

Table 5. Results of a χ^2 test comparing phytoplankton counts at the beginning and at the end of each experiment as a function of the submersion time of *S. cucullata*. Significant differences ($p<0.01$) are marked with *.

	Time submersed (h)											
	0.5	1	1.5	2	3	4	5	6	7	8	9	10
Nauplius copepod	2.38	0.63	1.49	0.71	160.26*	151.57*	159.29*	131.35*	117.56*	112.80*	116.24*	110.05*
Calanoid adult	0.10	0.06	0.31	0.17	0.09	0.08	0.43	14.06	19.10*	17.31*	12.52	11.41
Gastropod 2	0.23	0.12	0.23	0.49	12.73	9.45	8.24	7.69	7.60	6.61	6.41	7.43
Cal. j.+Cyc. j.+For.	0.07	0.16	0.36	0.32	9.17	12.66	12.76	9.71	7.85	8.62	7.22	9.09
Cyc.a.+Nem.	0.18	0.26	0.26	0.30	0.41	1.33	3.10	4.16	5.14	2.89	4.27	3.52
Oys.+Fis.+Gas.	0.28	0.52	0.53	2.11	3.56	3.81	9.04	7.33	11.69	8.63	5.74	8.70

Table 6. Results of a χ^2 test comparing zooplankton counts at the beginning and at the end of each experiment as a function of the submersion time of *S. cucullata*. Significant differences ($p<0.01$) are marked with *.

Genera	Spheric Equivalent Diameter (SED) (µm)			Percentage of the total phytoplankton carbon weight in the water (%)		
	Med.	Min.	Max.	Med.	Min.	Max.
Actinoptychus	78.1	40.5	97.1	11.0	7.6	14.7
Amphiprora	37.4	22.0	55.3	1.1	0.6	1.7
Amphora	30.7	20.6	42.8	1.2	0.8	1.7
Asterionella	26.6	21.2	39.0	0.1	0.0	0.2
Auricula	80.2	59.7	107.7	2.1	0.9	1.8
Bacillaria	9.9	5.0	13.2	0.1	0.0	0.1
Biddulphia	81.9	51.2	99.7	10.8	6.3	9.7
Chaetoceros	5.0	2.9	6.8	22.3	9.6	30.4
Climacosphenia	75.0	37.6	111.1	1.8	1.1	2.6
Coscinodiscus	36.8	24.6	47.7	1.8	1.4	2.6
Cyclotella	20.5	11.5	29.5	0.1	0.1	0.1
Diploneis	23.8	17.8	32.3	0.2	0.1	0.4
Grammatophora	35.4	24.4	46.9	1.7	0.3	4.8
Gyrodinium	19.5	15.1	24.3	2.4	1.1	7.4
Gyrosigma	50.8	39.5	71.9	2.3	1.9	3.1
Licmophora	60.2	43.0	82.6	0.4	0.2	0.8
Lymbia	45.0	32.0	64.3	1.8	1.0	3.0
Melosira	27.2	14.3	37.2	0.3	0.1	0.6
Merismopedia	31.2	21.1	44.2	0.1	0.1	0.2
Naviculla	28.0	21.7	35.8	13.1	6.2	21.4
Nitzschia	14.2	8.0	20.8	5.9	4.8	7.1
Nitzschiella	31.9	16.6	40.0	0.8	0.5	1.1
Oscillatoria	46.9	26.0	65.6	3.5	2.4	6.4
Peridinium	24.8	15.6	31.4	1.4	0.6	2.8
Pleurosigma	56.8	30.9	78.8	2.1	1.0	3.2
Rhabdonema	75.3	57.1	104.9	4.3	2.4	7.6
Rhizosolenia	58.1	41.6	77.2	0.8	0.2	1.6
Surirella	31.2	18.0	38.2	0.1	0.1	0.2
Triceratium	148.8	97.8	217.8	5.5	3.5	7.9

Table 7. Median, minimum and maximum spheric equivalent diameters of phytoplankton genera studied. The median, minimum and maximum percentages each genus is representing in the total phytoplankton carbon (at the beginning of the experiments) are also given.

would have been part of the DOC anyhow. The colloidal part is seen by the authors as being more closely related to the dissolved organic carbon than to the particulate organic carbon.

One difficulty with estimates of the uptake and clearance rates of particles from a fixed volume of medium is that the experimental organism is likely to be subject to a continuous decline in particle concentration with time, depending on the uptake and/or clearance rate and the volume of medium

	Spheric Equivalent Diameter (SED) (µm)			Percentage of the total phytoplankton carbon weight in the water (%)		
	Med.	Min.	Max.	Med.	Min.	Max.
Nauplius copepod	51.0	27.0	78.4	50.1	42.0	59.4
Calanoid adult	93.3	29.4	144.4	2.1	1.3	3.3
Calanoid juvenile	257.6	225.9	427.6	26.3	17.5	39.9
Cyclopoid adult	135.1	81.7	180.9	2.8	0.8	8.5
Cyclopoid juvenile	314.7	103.1	440.3	4.3	1.5	6.6
Gastropod 1	42.9	35.2	70.3	2.4	1.1	5.3
Gastropod 2	87.4	61.5	123.0	2.9	0.9	5.5
Oyster larvae	46.1	14.9	75.4	0.5	0.2	0.8
Fish egg	37.8	34.0	54.1	0.5	0.2	0.9
Nematode	218.2	141.5	354.0	3.6	1.9	6.0
Foraminifera	179.3	150.5	287.9	3.8	1.7	7.2

Table 8. Median, minimum and maximum spheric equivalent diameters of zooplankton groups studied. The median, minimum and maximum percentages each group is representing in the total zooplankton carbon (at the beginning of the experiments) are also given.

used. Coughlan (1969) suggested an exponential model for the calculation of the uptake and/or clearance rate from an observed decrease of particles in suspension. Although this method of calculation has been widely used in the past, Williams (1982) pointed out that it has serious drawbacks when applied to mixed particle spectra with differing retention efficiencies. Larger particles are likely to be removed first, leading to a progressive increase in the proportion of small particles and an apparent decline in the uptake/clearance rate. Similar objections apply to systems maintaining a constant concentration of particles (Winter, 1973) where a mixture of particles in the original proportions is added to an experimental medium which is subject to a preferential removal of the large size fraction. This problem can be minimised if a relative large volume of suspension medium is used, and if the system for measuring particle concentration is sufficiently sensitive to avoid the necessity of a major decrease in particle concentration with time. In the case of our measurements we were able to measure the uptake and clearance rates of a number of variables without a major decrease of the concentration of the variable studied (Table 3a, 3b, 4a and 4b).

As soon *S. cucullata* is submerged it will feed on relatively large amounts of DOC between 0.2 and 1.2 µm Stephens (1968) already stated dissolved organic carbon could be a substantial source of nutrition for marine organ-

isms.
The amount of dissolved organic carbon in the seawater used during our experiments is extremely high. Normally, seawater prefiltered through a 0.4 µm membrane filter contains about 3.6 mg DOC.l^{-1} (Flood *et al.*, 1992). Larger particles, up to 1 µm in diameter, normally account for at least 10% additional DOC (Koike *et al.*, 1990).
The mean amount of DOC <0.2 µm available in the seawater at the beginning of our experiments was 5.34 mg C.l^{-1} ± 2.09 mg C.l^{-1}. The mean amount of DOC between 0.2 and 1.2 µm was even higher: 11.77 mg C.l^{-1} ± 4.92 mg C.l^{-1}. Those values were similar to DOC values in other mangrove creeks (unpublished data).
High DOC values in mangrove waters, compared with other marine ecosystems, are probably related to detrital recycling within the mangrove ecosystem. The possibility exists the DOC concentration is also effected by the time of the year experiments were carried out. Experiments were carried out in the second part of August, just after the rainy season. Due to the rains a lot of sediments, nutrients, and probably dissolved organic carbon is brought to the coastal zone by the rivers.
It is not clear whether DOC in the size range of 0.2 to 1.2 µm is the only source of carbon during the first two hours. The Wilcoxon Matched Pairs Test comparing initial and end concentrations of the different food sources (Table 1 and 2) gives only significant differences for particles in this size range. However, the small number of replicas makes that this test will give a significant difference as soon all the oysters involved in the test are taking up food. The quantities do not matter.
If we compare the uptake and clearance rates of *S. cucullata* for DOC in the size range of 0.2 to 1.2 µm (Fig. 7) with other food sources, it is clear that DOC >0.2 and <1.2 µm is the most important food source of *S. cucullata* during the first hours it is submerged. Uptake and clearance rates of *S. cucullata* for the other food sources, including DOC <0.2 µm are negligible compared with the uptake and clearance rates of *S. cucullata* for DOC in the size range of 0.2 to 1.2 µm. The uptake and clearance rates of *S. cucullata* for two subelements of the DOC in the size range of 0.2 to 1.2 µm were examined: colloidal material and bacteria.
Figure 8a and b show the uptake and clearance rates of *S. cucullata* for colloidal melanin. The uptake and clearance rates follow more or less the same pattern as the uptake and clearance rates of *S. cucullata* for DOC between

0.2 and 1.2 µm. However, it is difficult to relate our results on colloidal melanin uptake to actual removal rates of colloidal material by *S. cucullata*. The size distribution and the shape of naturally occurring colloidal particles, as well as their overall quantity as a subfraction of DOC, is only partly known (Koike *et al.*, 1990). If we assume that at the same time *S. cucullata* takes up colloidal melanin, similar amounts of natural occurring colloidal DOC are taken up than the real uptake rate of colloidal material would account for more than 90% of the total DOC uptake of *S. cucullata*. There would be no change in clearance rate. Even with the most conservative hypothesis (uptake of colloidal melanin alone) the uptake rate of colloidal material accounts for almost 50% of the total DOC uptake rate.

The ability to take up colloidal 'particles' was shown for oikopleurids. These are appendicularian tunicates, often occurring in high densities in discrete strata at various depths. Under such conditions they may clear 30 to 60% of the water mass in 24 h (Alldredge, 1981). Obviously, they may repack colloidal DOC (>0.2 µm particle size) rapidly under such conditions (Flood *et al.*, 1992). On the basis of filter parameters this ability to graze on colloidal DOC is probably shared by caddisfly larvae (Wallace and Malas, 1976), pedal worms (Flood and Fiala-Medioni, 1982), ascidians (Flood and Fiala-Medioni, 1981), salps (Bone *et al.*, 1991) and amphioxus (Flood, 1981). Tack and Polk (1996) showed the uptake of colloidal melanin from seawater by marine bivalves in tropical and non-tropical marine habitats. They showed a high uptake of colloidal melanin for mangrove and seagrass meadow species, contrary to a low uptake for coral reef species.

The colloidal ink particles were ingested by *S. cucullata*, as evidenced by its blackened gut contents. Thanks to the high stability of natural occurring melanin biopolymers, we found microscopical evidence of cellular uptake of colloidal DOC by *S. cucullata* (Fig. 12).

The uptake and clearance rates of *S. cucullata* for bacteria follows the same trend as the uptake and clearance rates for DOC in general. During the first two hours the uptake rate for bacteria represents only 10% of the total uptake rate for DOC. The uptake rate for colloidal particles is 6 times higher than the uptake rate for bacteria. However the differences between the clearance rates for bacteria and colloidal DOC show a clear preference of *S. cucullata* for the bacterial food component. Those results are consistent with the results of Wright *et al.* (1982). They showed the efficient clearance of natural bacterioplankton by the saltmarsh mussel *Geukensia demissa*. The very

high clearance rate explains the results of Mwangi (1994) who found a reduction of bacterial biovolume in a mangrove creek due to *S. cucullata*.
After *S. cucullata* is submerged for two hours the uptake rate for DOC decreases until it stabilises around the sixth hour at a value between 0.1 and 2.5 mg $C.h^{-1}.ind^{-1}$. Two hours after submersion the uptake rate of *S. cucullata* for POC starts to increase. At a maximum submersion time of 10 hours the uptake rate for DOC is still higher than the uptake rate for POC but both are in the same order of magnitude. The preferential uptake of DOC during the first three hours changes between the third and fourth hour after submersion into a preferential uptake of POC. Even with similar uptake rates of *S. cucullata* for DOC and POC when the oyster is submerged for more than 9 hours there is a clear preference of *S. cucullata* to take up POC at that moment.
Phytoplankton is a minor contributor to the global uptake rate of *S. cucullata* for POC. Zooplankton on the other hand explains the uptake rate for POC for almost 100% up to a submersion time of seven hours. Since *S. cucullata* shows no preferential clearance for either zoo or phytoplankton the differences between the uptake rates of *S. cucullata* for zoo and phytoplankton can be explained by the different concentrations of those food sources in the seawater. The steep increase in uptake and clearance rates for POC from the seventh hour onwards can be explained by the uptake of large seston particles.
The clearance rate for phytoplankton increases very fast between 1.5 and 3 hours, while the clearance rate for zooplankton increases between 2 and 7 hours after *S. cucullata* is submerged.
If we study the relation between the mean diameter of each plankton genus and the time at which a first significant difference (χ^2) is seen between the initial and end concentrations (counts/600 ml) of both zoo and phytoplankton (Fig. 10) we see a clear correlation between both variables. The uptake of the smallest particles (5 µm) starts already within the first 30 min. The uptake of the largest particles (260 µm) is seen after the sixth hour *S. cucullata* is submerged.
An overview of the uptake and clearance rates of *S. cucullata* for the different carbon sources is shown in Figure 13a and b. The main food source of *S. cucullata* seems to be DOC in the size range between 0.2 and 1.2 µm. During the first two hours *S. cucullata* is submerged, it prefers to clear DOC in the size range between 0.2 and 1.2 µm out of the water. Between a submersion time of two and four hours *S. cucullata* shows no clear preference in the

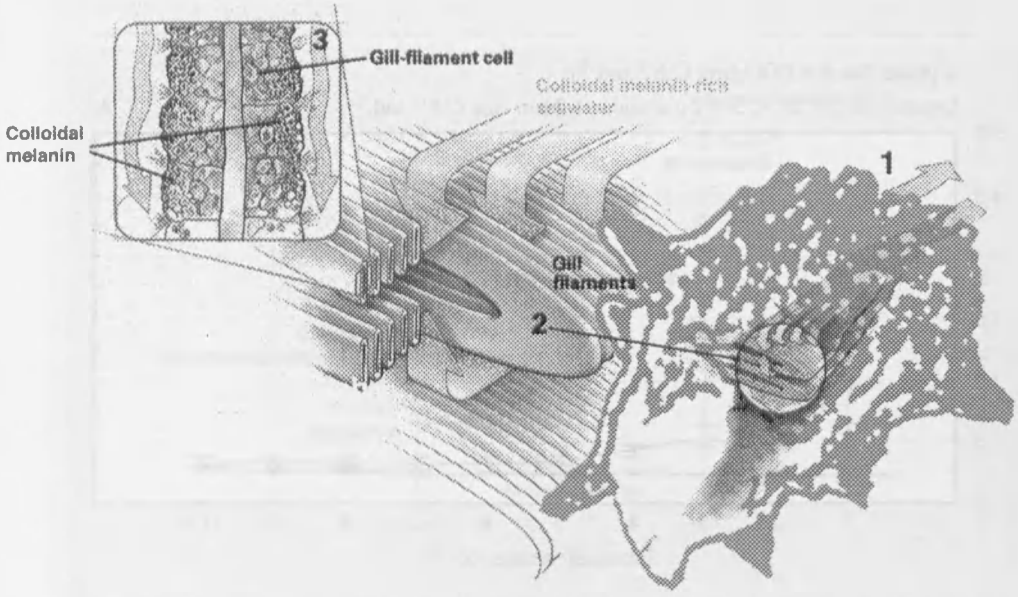

Figure 12. Cocked open, *Saccostrea cucullata*: draws water in and pushes it out (1). Inside the shells - about 8 cm long when fully grown - water washes over hundreds of gill filaments (2). Cellular uptake of colloidal melanin (3) seems to be an important food source of *S. cucullata*.

uptake of DOC or POC. After a submersion time of more than four hours *S. cucullata* shows a clear preference to feed on POC. However, even then are the actual amounts of POC taken up by *S. cucullata* relative small compared with the uptake of DOC.

The importance of DOC between 0.2 and 1.2 µm brings us to the question where this DOC is coming from. Comparing $\delta^{13}C$ values of *S. cucullata* for different submersion times (Fig. 11) with values of possible food sources (Fig. 14) can give us an indication. The $\delta^{13}C$ values of *S. cucullata* range between -19‰ and -23‰. Compared with the $\delta^{13}C$ values of different compartments of the mangrove ecosystem (Woitchik *et al.*, 1993) we find seagrasses ($\delta^{13}C$ between -9.92‰ and -20.22‰) and copepods ($\delta^{13}C$ between -20.09‰ and -24.10‰) as possible carbon sources of *S. cucullata*. The $\delta^{13}C$ value of the copepods can explain why the $\delta^{13}C$ value of the oysters increas-

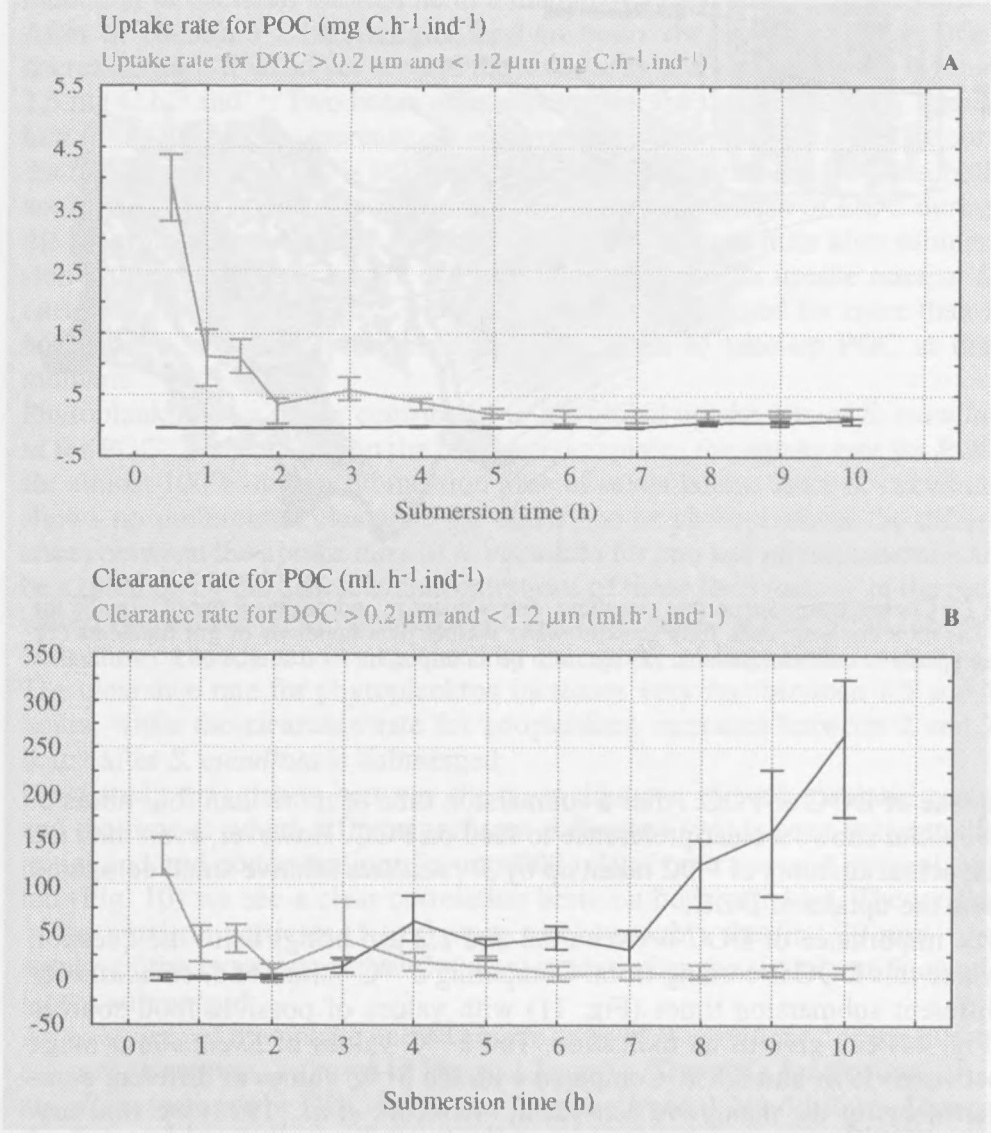

Figure 13. Saccostrea cucullata:: uptake rates (mg $C.h^{-1}.ind^{-1}$) and clearance rates ($ml.h^{-1}.ind^{-1}$) of POC and DOC in function of its submersion time (h).

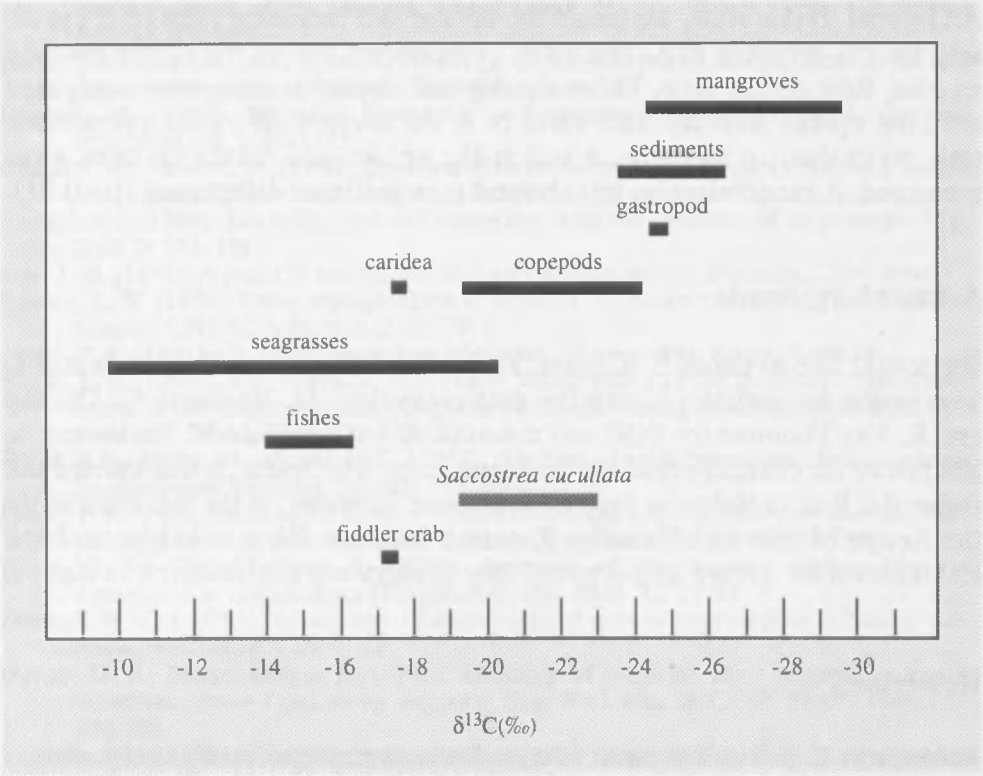

Figure 14. $\delta^{13}C$ values (‰) in different components of the mangrove ecosystem in Gazi Bay (after Woitchik *et al.*, 1993).

es with the total submersion time. On the other hand this study has shown the relative low uptake of POC, even after long submersion times. Mangroves with a $\delta^{13}C$ value between -24.28‰ and -29.71‰ can be another food source for oysters with long submersion times.

The uptake of DOC by *S. cucullata* within the first three hours coincides with specimens with $\delta^{13}C$ values comparable with those of seagrasses. The increasing $\delta^{13}C$ values, related to increased submersion times, show a shift towards the uptake of copepods and mangrove detritus. The uptake of mangrove detritus was confirmed after microscopical analyses of the larger seston particles.

Additional to this study we tested the uptake and clearance rates of *S. cucullata* for the different POC and DOC components before the oyster reached its total submersion time. Those uptake and clearance rates were compared with the uptake and clearance rates of *S. cucullata* with a total submersion time equivalent to the time at which the uptake and clearance rates were measured. A randomisation test showed no significant differences ($p < 0.01$).

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References

Alldredge, A. L. (1981). The impact of appendicularian grazing on natural food concentrations in situ. Limnol. Oceanogr. 26: 247-257

Bagnara, J. T., Hadley, M. E. (1973). Chromatophores and color change, the comparative physiology of animal pigmentation. Prentice-Hall, Englewood Cliffs

Barille, L., Prou, J., Heral, M., Bourgrier, S. (1993). No influence of food quality, but ration-dependent retention efficiencies in the Japanese oyster *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. 171: 91-106

Bayne, B. L., Newell, R. C. (1984). Physiological energetics of marine molluscs. In: Saleuddin, A. S. M., Wilbur, K. M. (Ed.) The Mollusca, Vol. 4 Physiology Part 1. Academic Press, New York, p. 407-515

Bayne, B. L., Thompson, R. J., Widdows, J. (1976a). Physiology 1. In: Bayne, B. L. (Ed.) Marine mussels, their ecology and physiology. Cambridge University Press, Cambridge, p. 121-206

Bayne, B. L., Widdows, J., Thompson, R. J. (1976b). Physiology 2. In: Bayne, B. L. (Ed.) Marine mussels, their ecology and physiology. Cambridge University Press, Cambridge, p. 207-260

Berry, P. F., Schleyer, M. M. (1983). The brown mussel *Perna perna* on the Natal coast, South Africa: utilization of available food and energy budget. Mar. Ecol. Prog. Ser. 13: 201-210

Bollen, A. (1993). *Saccostrea cucullata* (von Born, 1778): Voedselaanwezigheid in een

mangrovekreek (Gazi, Kenya). Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels
Bone, Q., Braconnot, J.-C., Ryan, K. P. (1991). On the pharyngeal feeding filter of the salp *Pegea confoederata* (Tunicata: Thaliacea). Acta Zool. 72: 55-60
Bratbak, G. (1985). Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol. 48: 1488-1493
Chelazzi, G., Vannini, M. (1980). Zonation of intertidal molluscs on rocky shores of southern Somali. Estuar. coast. mar. Sci. 10: 569-583
Coughlan, J. (1969). The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2: 356-358
Day, J. H. (1974). A guide to marine life of South African shores. Balkema, Cape Town
Eppley, R. W. (1978). From phytoplankton to biomass. In: Sournia, A. (Ed.) Phytoplankton manual. UNESCO, Paris, p. 273-279
Flood, P. R. (1981). On the ultrastructure of mucus. Biomed. Res. Suppl. 2: 49-53
Flood, P. R., Deibel, D., Morris, C. C. (1990). Visualization of the transparent gelatinous house of the pelagic tunicate *Oikopleira vanhoeffeni* using *Sepia* ink. Biol. Bull. mar. biol. Lab. Woods Hole 178: 118-125
Flood, P. R., Deibel, D., Morris, C. C. (1992). Filtration of colloidal melanin from sea water by planktonic tunicates. Science 355: 630-632
Flood, P. R., Fiala-Medioni, A. (1981). Ultrastructure and histochemistry of the food trapping mucous film in benthic filter-feeders (Ascidians). Acta Zool. 62: 53-65
Flood, P. R., Fiala-Medioni, A. (1982). Structure of the mucous feeding filter of *Chaetopterus variopedatus* (Polychaeta). Mar. Biol. 72: 27-33
Hartnoll, R. G. (1976). The ecology of some rocky shores in tropical East Africa. Estuar. coast. mar. Sci. 4: 1-21
Haven, D. S., Mores-Alamo, R. (1970). Filtration of particles from suspension by the American oyster *Crassostrea virginica*. Biol. Bull. mar. biol. Lab. Woods Hole 139: 248-264
Hildreth, D. I. (1980). Bioseston production by *Mytilus edulis* and its effect in experimental systems. Mar. Biol. 55: 309-315
Jørgensen, C. B. (1960). Efficiency of particle retention and rate of water transport in undisturbed lamellibranchs. J. Cons. Int. Explor. Mer 26: 94-116
Jørgensen, C. B. (1975a). Comparative physiology of suspension feeding. Annu. Rev. Physiol. 37: 57-79
Jørgensen, C. B. (1975b). On gill function in the mussel *Mytilus edulis* L. Ophelia 13: 187-232
Jørgensen, C. B. (1983). Fluid mechanical aspects of suspension feeding. Mar. Ecol. Prog. Ser. 11: 89-103
Koike, I., Shigemitsu, H., Kazuki, T., Kazuhiro, K. (1990). Role of sub-micrometre particles in the ocean. Science 345: 242-244
LaBarbera, M. (1978). Particle capture by a Pacific brittle star: experimental test of the aerosol suspension feeding model. Science 201: 1147-1149
Lewis, J. R. (1964). The ecology of rocky shores. Hodder & Stoughton, London
Loosanoff, V. L. (1949). On the food selectivity of oysters. Science 110: 122
Manahan, D. T. (1983). The uptake and metabolism of dissolved amino acids by bivalve larvae. Biol. Bull. 164: 236-250
Manahan, D. T., Wright, S. H., Stephens, G. C., Rice, M. A. (1982). Transport of Dissolved Amino Acids by the Mussel, *Mytilus edulis*: Demonstration of Net Uptake from

Natural Seawater. Science 215: 1253-1255
Møhlenberg, F., Riisgard, M. V. (1978). Efficiency of particle retention in 13 species of suspensionfeeding bivalves. Ophelia 17: 239-246
Mwangi, S. N. (1994). The influence of bivalve filter feeders on bacteria populations and seston in Gazi Bay and Wadden Sea. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels
Newell, R. I. E., Jordan, S. J. (1983). Preferential ingestion of organic material by the American oyster, *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 13: 47-53
Okemwa, E. R., Ruwa, R. K., Polk, P. (1986). The autecology of the edible oyster *Crassostrea cucullata* Born, 1778: size-related vertical distribution at Mkomani, Mombasa. J. Sci. Ser. B 7: 9-14
Palmer, R., Williams, L. (1980). Effect of particle concentration on filtration efficiency by the bay scallop *Argopecten iradians* and the oyster *Crassostrea virginica*. Ophelia 19: 163-174
Peirson, W. M. (1983). Utilization of eight algal species by the bay scallop, *Argopecten irradians concentricus* (Say). J. Exp. Mar. Biol. Ecol. 68: 1-11
Rubenstein, D. E., Koehl, M. A. R. (1977). The mechanisms of filter-feeding: some theoretical considerations. Am. Nat. 111: 981-994
Ruwa, R. K. (1984). Invertebrate faunal zonation on rocky shores around Mombasa, Kenya. Kenya J. Sci. Ser. B 5: 49-65
Ruwa, R. K. (1990). Growth of *Crassostrea cucullata* Born (Bivalvia) at different levels in the intertidal zone. Aquaculture 88: 303-312
Shumway, S. E., Cucci, T. L., Newell, R. C., Yentsch, C. M. (1985). Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Biol. Ecol. 91: 77-92
Silvester, N. R., Sleigh, M. A. (1984). Hydrodynamic aspects of particle capture by *Mytilus*. J. Mar. Biol. Assoc. U.K. 64: 859-879
Stephens, G. C. (1968). Dissolved Organic Matter as a Potential Source of Nutrition for Marine Organisms. Am. Zool. 8: 95-106
Strickland, J. D. H., Parsons, T. R. (1972). A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottawa
Tack, J. F. (1992). Ecomorphology of the mangrove oyster *Saccostrea cucullata* (von Born, 1778). Academiae Analecta 54 (4): 1-20
Tack, J. F., Polk, P. (1996). The Uptake of Colloidal Melanin from Seawater by Marine Bivalves. Mar. Ecol. 17: 543-548
Tack, J. F., Vanden Berghe, E., Polk, P. (1992). Ecomorphology of *Crassostrea cucullata* (Born, 1778) (Ostreidae) in a mangrove creek (Gazi, Kenya). Hydrobiologia 247: 109-117
Taylor, J. D. (1968). Coral reef associated invertebrate communities (mainly molluscan) around Mahe, Seychelles. Phil. Trans. r. Soc., Lond. Ser. B 254: 129-206
Taylor, J. D. (1970). Intertidal zonation at Aldabra atoll. Phil. Trans. r. Soc., Lond. Ser. B 260: 173-213
Urban, E. R., Kirchman, D. L. (1992). Effect of kaolinite clay on the feeding activity of the eastern oyster *Crassostrea virginica* (Gmelin). J. Exp. Mar. Biol. Ecol. 160: 47-60
Vahl, O. (1972a). Efficiency of particle retention in *Mytilus edulis* L. Ophelia 10: 17-25
Vahl, O. (1972b). Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* (L.) (Bivalvia). Ophelia 10: 67-74
Vahl, O. (1973a). Porosity of the gill, oxygen consumption and pumping rate in *Cardium edule* (L.) (Bivalvia). Ophelia 10: 109-118

Vahl, O. (1973b). Efficiency of particle retention in *Chlamys islandica* O.F. Müller. Astarte 6: 21-25

Van Evelghem, K. (1996). Opname van glucose bij *Saccostrea cucullata* (von Born, 1778) en fytoplankton. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Wallace, B. J., Malas, D. (1976). The significance of the elongate rectangular mesh found in capture nets of fine particle filter feeding trichoptera larvae. Arch. Hydrobiol. 77: 205-212

Williams, L. G. (1982). Mathematical analysis of the effects of particle retention efficiency on determination of filtration rate. Mar. Biol. 66: 171-177

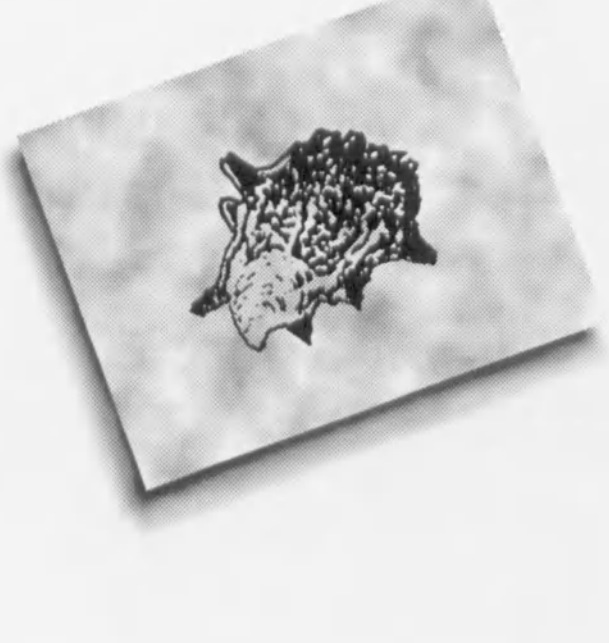
Winter, J. E. (1973). The filtration of *Mytilus edulis* and its dependence on algal concentration, measured by a continuous automatic recording apparatus. Mar. Biol. 22: 317-328

Winter, J. E. (1978). A review of the knowledge of suspension-feeding in lamellibranchiata bivalves, with special reference to artificial aquaculture systems. Aquaculture 13: 1-33

Woitchik, A. F., Kazungu, J., Dehairs, F. (1993). Transfer of C from primary to secondary and tertiary producers: preliminary results. In: Woitchik, A. F. (Ed.) Final Report Dynamics and Assessment of Kenyan Mangrove Ecosystems. ANCH, Vrije Universiteit Brussel, Brussels, p. 216-220

Wright, R. T., Coffin, R. B., Ersing, C. P., Pearson, D. (1982). Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27: 91-98

Wright, S. H. (1982). A Nutritional Role for Amino Acid Transport in Filter-Feeding Marine Invertebrates. Am. Zool. 22: 621-634



Chapter

5

SETTLING BEHAVIOUR

Spawning behaviour and larval culture of *Saccostrea cucullata* (von Born, 1778)

Abstract

Saccostrea cucullata (von Born, 1778) larvae from naturally spawned oysters were grown to settlement within a hatchery environment. Significant differences were observed in spawning efficiency among adult oysters kept in seawater of different salinities. The lower the salinity, the higher the spawning efficiency. When fed a mixture of marine diatoms (*Chaetoceros sp.* and *Nitzschia sp.*) the whole larval period, from fertilised egg to metamorphosis takes nearly 28 days. Once the larvae reach the straight-hinge stage they have the tendency to sink to the bottom of the tank. Salinity is the most important factor influencing the upward movement of *S. cucullata* larvae just before settlement.

Introduction

Scientific studies on the feeding and settling behaviour of oyster larvae (Douillet, 1993; Fitt *et al.,* 1989, 1990; Tamburri *et al.,* 1992; Zimmer-Faust and Tamburri, 1994) are depending on the availability of large quantities of oyster larvae. Also the commercial cultivation of oysters depends in many cases on the supply of young oysters (spat) by hatcheries (Chew, 1990). Hatcheries produce spat in large quantities on demand. Oyster spat produced at hatcheries has a number of advantages compared with spat collected from

the wild: substrate-free (Dupuy and Rivkin, 1972; Hidu *et al.,* 1981; Coon *et al.,* 1986), triploid (Stanley *et al.,* 1984; Chaiton and Allan, 1985; Allen and Downing, 1986) and/or selective bred traits (Newkirk, 1980).
Larval culture of bivalve molluscs is well established (Loosanoff and Davis, 1963; Dupuy *et al.,* 1977; Castagna and Kraeuter, 1984). Larval culture of *Crassostrea rhizophorae* (Guilding) is described by Rampersad and Ammons (1992). *C. rhizophorae*, commonly known as the mangrove oyster, grows on the aerial roots of mangrove trees of Central America, South America and the Caribbean. The successful mating between *C. rhizophorae* and *Crassostrea virginica* has led to the suggestion that *C. rhizophorae* is a subspecies of *C. virginica* (Menzel, 1987).
Saccostrea cucullata (von Born, 1778), inhabiting the mangrove forests bordering the Indian Ocean, is closely related to *C. rhizophorae*. In the eighties the fast increase in tourism led to an overfishing of *S. cucullata*, leading to a shortage of large specimens. In 1985 the Kenya Belgium Project in Marine Sciences, a scientific co-operation between Brussels Free University and the Kenya Marine and Fisheries Research Institute initiated scientific research on culturing techniques (Okemwa *et al.,* 1986; Ruwa, 1990; Tack, 1992; Tack *et al.,* 1992) and aquacultural potential (Poznanski, 1989; Goyvaerts, 1995) of *S. cucullata*. To date, the Kenya Belgium Project in Marine Sciences runs two commercial oyster cultures along the Kenyan coast. One of them the largest rack culture on the African continent. The culture of *S. cucullata* larvae is not yet reported.
In this study we investigate the feasibility to induce spawning by *S. cucullata* at different temperatures and salinities. We also attempted to produce spat from *S. cucullata* larvae under controlled conditions. We report the successful rearing of *S. cucullata* larvae to settlement.

Material and methods

Spawn inducement

Adult oysters were collected from mangrove trees (*Rhizophora mucronata*) in a mangrove area adjacent to Gazi, a village approximately 50 km south of Mombasa (Kenya). Brood oysters were removed from the water at least six hours prior to inducement. They were transported out of the water to the lab-

oratory of the Kenya Marine and Fisheries Research Institute. Oysters were placed in approximately 5 cm deep seawater with a constant pH (6.8) but different temperatures (ranging between 24 and 31 °C) and salinities (ranging between 18 and 33‰). The animals were not fed any supplementary food. After 72 hours an egg solution obtained from stripped females was added. Male oysters subsequently spawned, followed by females. As soon female oysters started spawning they were removed to individual 500 ml beakers and allowed to finish the spawn. Eggs were found to be fertilised and were used without further exposure to sperm.

Larval rearing

All larvae were obtained from the spawn of a single female kept at a temperature of 30 °C and at 24‰ salinity. Larvae were reared in a 50 l tank. They were kept at 0.5 to 1.0 larvae.ml^{-1} in natural seawater at the same salinity, pH and temperature as the adult 'mother' oyster with a 12:12 dark:light cycle (light on: 06.30 a.m.). Prior to use, the natural seawater was filtered over a 0.22 µm filter and autoclaved for 15 min at 150 °C and 15 psi. Cultures were aerated by air bubbled through Pasteur pipettes. The culture medium was changed daily to preclude the build up of pathogenic bacteria (Loosanoff and Davis, 1963). The oyster larvae were fed daily with a mixture of marine diatoms (*Chaetoceros sp.* and *Nitzschia sp.*) at 2.5 x 10^4 cells.ml^{-1}.

Setting

Larvae caught on a 300 µm screen were removed to a holding tank and reared as before until the larvae developed pigmented eye spots. Larvae were then transferred to a setting tank containing desired setting substrate.

Results

The total number of fertilised eggs taken from the spawn of a single female ranged between 22,000 and 330,000. At constant temperatures we can see a clear decrease in the number of fertilised eggs with increasing salinities (Fig. 1). Figure 2 shows the number of fertilised eggs at constant salinities, but at

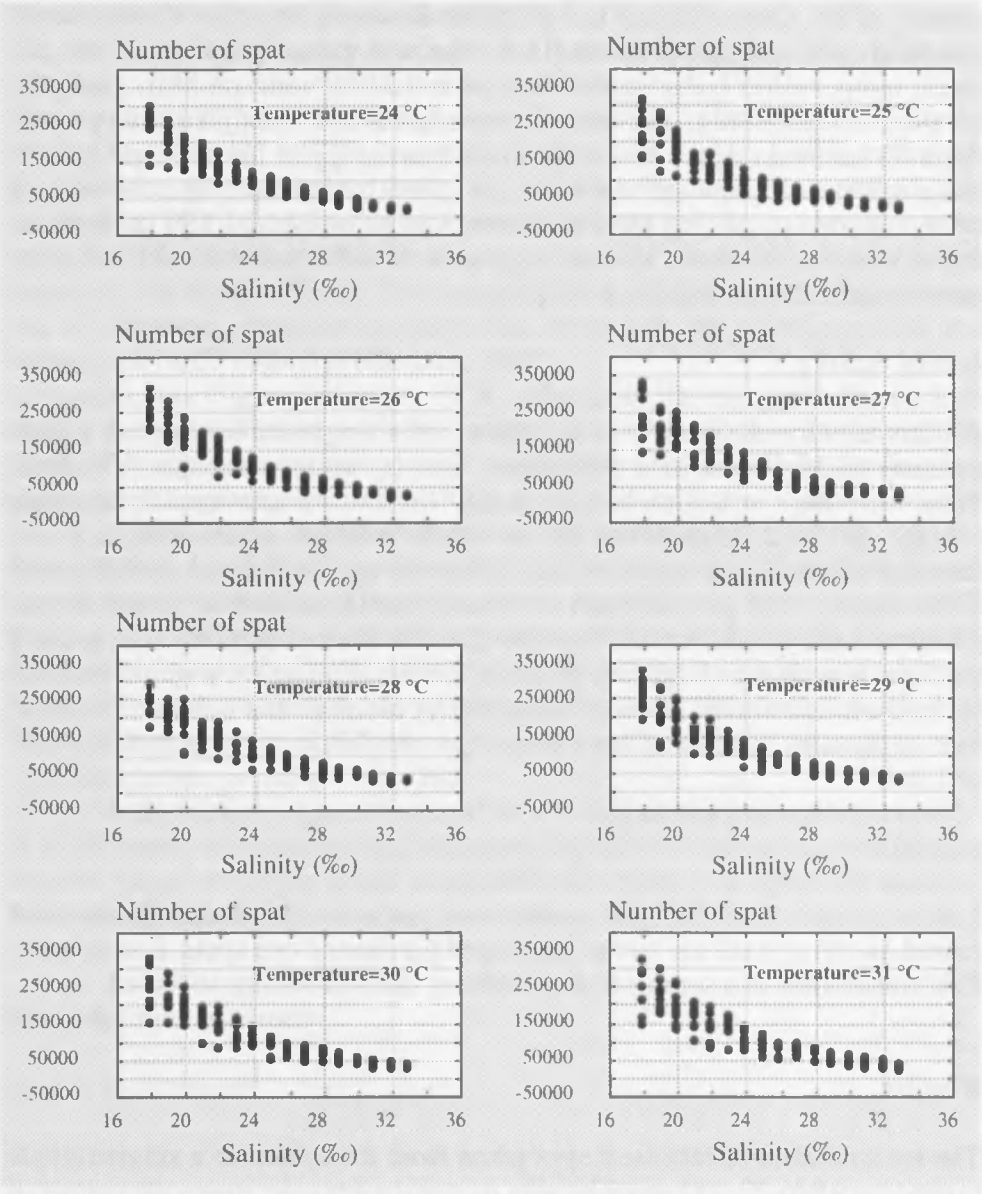


Figure 1. S. cucullata: number of oyster spat as a function of the salinity (‰) for different temperatures

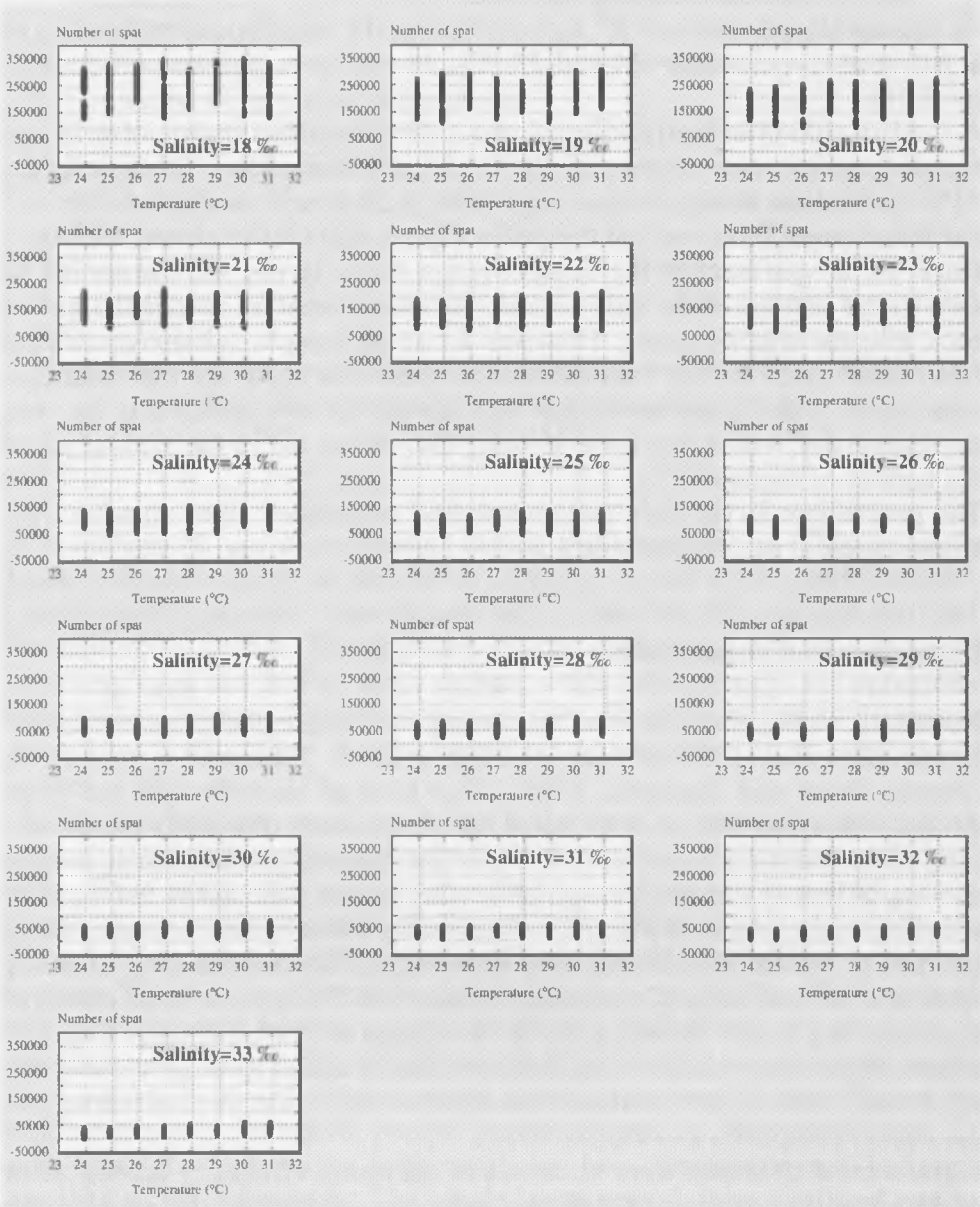


Figure 2. S. cucullata: number of oyster spat as a function of the temperature (°C) for different salinities

increasing temperatures. A χ^2 test comparing the number of fertilised eggs at extreme temperatures (24 and 33 °C) showed no significant differences ($p<0.01$).
A total of 100,000 fertilised eggs was taken from the spawn of a single female kept at a temperature of 30 °C and at 24‰ salinity. Of those eggs, 74% reached the straight-hinge stage (after ± 24 hours) and 53% of the initial larval population reached the pediveliger stadium after almost 26 days.
Once the larvae reached the straight-hinge stage, larvae were observed to sink to the bottom of the culture tank. Increased aeration forced the larvae back into the water column. However a sudden drop in salinity by adding fresh water to the culture medium brought the larvae back into the water column, even without any aeration. This change in swimming activity was immediate and would last for ± 30 min, after which the larvae sank again to the bottom of the tank.
The pediveliger larvae only metamorphosed and settled when an adult oyster was present in the setting tank.

Discussion and conclusions

Numerous authors have studied the settling and feeding behaviour of oyster larvae (Douillet, 1993; Fitt *et al.,* 1989, 1990; Tamburri *et al.,* 1992; Zimmer-Faust and Tamburri, 1994). This kind of research asks for large amounts of oyster larvae of the same age. In function of a study on the settling behaviour of *S. cucullata* we tested the feasibility to induce spawning and to produce spat from *S. cucullata* larvae. Larvae were easily obtained by inducing adult oysters to liberate their gametes. Male oysters appeared more inclined to spawn when induced by either egg or sperm. Inducing males to spawn, which subsequently induced females was found to be more effective than inducing female oysters directly by a sperm solution. All eggs were fertilised, even when released in a container free of added sperm. Probably the eggs were fertilised by sperm collected by the adult prior to its placement in the beaker (Stephano and Gould, 1988).
Significant differences were observed in spawning efficiency among adult oysters kept in seawater of different salinities. The lower the salinity, the higher the spawning efficiency. Salt stress is probably the explanation. The spawning efficiency did not change in function of the temperature. In natur-

Developmental stage	Time after fertilisation
Formation of fertilisation membrane	5 min
Release of 1st polar body	40 min
Release of 2nd polar body	45 min
1st cleavage	1 h 40 min
2nd cleavage	1 h 50 min
Gastrule	5 h
Trochophore	10 to 12 h
Straight-hinge stage	20 to 25 hours

Table 1. Early development in *S. cucullata* larvae.

al conditions *S. cucullata* spawns during the rainy season. This certainly leads to an increase of the number of larvae produced by the adult oysters.
Larval development of *S. cucullata* was described by Kalyanasundaram and Ramamoorthi (1987). The diameter of the unfertilised egg is ± 40 µm. Maturing eggs are oval or flask shaped, while matured eggs are spherical. The nucleus is comparatively large and very clear and begins to disappear soon after fertilisation. The chronological development of *S. cucullata* is shown in Table 1.
At the straight-hinge stage, the velum is the main locomotion organ. Using their cilia, the larvae swim actively in all directions and crowd near the surface of the water. The earliest straight-hinge larvae are about 65 µm in length. They retain their 'D' shape until they reach 80-85 µm in length. During this stage the height increases from 60 to 80 µm and length increases from 70 to 85 µm. The umbo starts to form on the 7th day. The length of the oyster larvae is by then approximately 90 µm. The shape of the umbo is knobbly. Length reaches ± 140 µm on the 11th day. The foot develops on the 26th day when the pediveliger reaches a length between 300 and 330 µm. At that moment the eye spot is present but it disappears during metamorphosis into spat. The pediveliger metamorphoses into plantigrade and becomes attached to the substratum. The whole larval period, from fertilised egg to metamorphosis takes nearly 28 days.
Once the larvae reach the straight-hinge stage they have the tendency to sink

to the bottom of the tank. This probably explains the low spheric equivalent diameter of oyster larvae found in plankton samples in Gazi Bay (Bollen, 1993). According to Galtsoff (1964) bivalve larvae in estuaries were found especially near to the bottom of the water column. An upward movement in the column coincided with tidal changes. Galtsoff (1964) suggested these changes in vertical position maintained the larvae in the estuary. Our results suggest that salinity is the most important factor influencing the vertical movement of *S. cucullata* larvae. This is consistent with high settlement of *S. cucullata* larvae immediate after rainfall and during the rainy seasons. Settling of larvae was immediate after exposure of the pediveliger larvae to an adult *S. cucullata* specimen. We assume that one or more substances originating from the adult oysters triggered settling.

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References

Allen, S. K., Downing, S. L. (1986). Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content and sexual maturation in yearlings. J. Exp. Mar. Biol. Ecol. 102: 197-208

Bollen, A. (1993). *Saccostrea cucullata* (von Born, 1778): Voedselaanwezigheid in een mangrovekreek (Gazi, Kenya). Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Castagna, M., Kraeuter, J. N. (1984). Manual for growing the hard clam *Mercenaria*. Special Report in Applied Marine Science and Ocean Engineering No. 249, Virginia Institute of Marine Science, Gloucester Point, Virginia

Chaiton, J. A., Allen, S. K. (1985). Early detection of triploidy in the larvae of Pacific oysters, *Crassostrea gigas*, by flow cytometry. Aquaculture 48: 35-43

Chew, K. K. (1990). Global bivalve shellfish introduction. World Aquacult. 21: 9-22

Coon, S. L., Bonar, D. B., Wiener, R. M. (1986). Chemical production of clutchless oyster spat using epinephrine and norepinephrine. Aquaculture 58: 255-262

Douillet, P. A. (1993). Carbon contribution through bacterivory in larvae of the Pacific oyster *Crassostrea gigas*. Mar. Ecol. Prog. Ser. 102: 303-314

Dupuy, J. L., Rivkin, S. (1972). The development of laboratory techniques for the produc-

tion of clutch-free spat of the oyster *Crassostrea virginica*. Chesapeake Sci. 13: 45-52

Dupuy, J. L., Windsor, N. T., Sutton, C. (1977). Manual for the design and operation of an oyster seed hatchery. Special Report in Applied Marine Science and Ocean Engineering No. 142, Virginia Institute of Marine Science, Gloucester Point, Virginia

Fitt, W. K., Coon, S. L., Walch, M., Weiner, R. M., Colwell, R. R., Bonar, D. B. (1990). Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. Mar. Biol. 106: 389-394

Fitt, W. K., Labare, M. P., Fuqua, W. C., Walch, M., Coon, S. T., Bonar, D. B., Colwell, R. R., Weiner, R. M. (1989). Factors influencing bacterial production of inducers of settlement behavior of larvae of the oyster *Crassostrea gigas*. Microb. Ecol. 17: 287-298

Galtsoff, P. S. (1964). The American oyster *Crassostrea virginica* Gmelin. Fish. Bull. US Fish Wildl. Serv. 64: 369-371

Goyvaerts, E. (1995). Commercial Oyster Farming Along the Kenyan Coast - a Case Study on Sustainable Exploitation of a Natural Resource and Community Development. Unpublished. M. Sc. Thesis, Concordia International University, USA

Hidu, H., Chapman, S. R., Dean, D. (1981). Oyster mariculture in subboreal (Maine, United States of America) waters: clutchless setting and nursery culture of European and American oysters. J. Shellfish Res. 1: 57-67

Kalyanasundaram, M., Ramamoorthi, K. (1987). Larval development of the oyster *Saccostrea cucullata* (Born). Mahasagar Bull. of the Nat. Inst. of Oceanogr. 20: 53-58

Loosanoff, V. L., Davis, J. C. (1963). Rearing of bivalve mollusks. In: Russell, F. S. (Ed.) Advances in Marine Biology. Academic Press, London, p. 1-136

Menzel, W. (1987). Hybridization of oysters and clams. In: Proceedings of the World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture. Heenemann, Berlin, p. 48-59

Newkirk, G. F. (1980). Review of the genetics and the potential for selective breeding of commercially important bivalves. Aquaculture 19: 209-228

Okemwa, E. R., Ruwa, R. K., Polk, P. (1986). The autecology of the edible oyster *Crassostrea cucullata* Born, 1778: size-related vertical distribution at Mkomani, Mombasa. J. Sci. Ser. B 7: 9-14

Poznanski, M. (1989). Evaluation d'un projet ostréicole sur la côte kenyane. Unpublished. M. Sc. Thesis, Université Libre de Bruxelles, Brussels

Rampersad, J. N., Ammons, D. R. (1992). Production of *Crassostrea rhizophorae* (Guilding) spat from hatchery-reared larvae. Aquaculture 106: 253-260

Ruwa, R. K. (1990). Growth of *Crassostrea cucullata* Born (Bivalvia) at different levels in the intertidal zone. Aquaculture 88: 303-312

Stanley, J. G., Hidu, H., Allen, S. K. (1984). Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. Aquaculture 37: 147-155

Stephano, J. L., Gould, M. (1988). Avoiding polyspermy in the oyster (*Crassostrea gigas*). Aquaculture 73: 295-307

Tack, J. F. (1992). Ecomorphology of the mangrove oyster *Saccostrea cucullata* (von Born, 1778). Academiae Analecta 54 (4): 1-20

Tack, J. F., Vanden Berghe, E., Polk, P. (1992). Ecomorphology of *Crassostrea cucullata* (Born, 1778) (Ostreidae) in a mangrove creek (Gazi, Kenya). Hydrobiologia 247: 109-117

Tamburri, M. N., Zimmer-Faust, R. K., Tamplin, M. L. (1992). Natural Sources and Properties of Chemical Inducers Mediating Settlement of Oyster Larvae: A Re-examination. Biol. Bull. 183: 327-338

Zimmer-Faust, R. K., Tamburri, M. N. (1994). Chemical identity of oyster larval settlement cues. J. Shellfish Res. 13: 282

Chemical inducers mediating settlement of *Saccostrea cucullata* (von Born, 1778) larvae

Abstract

In this study live adult oysters and biofilms were separated as possible source of waterborne chemical inducers of settling behaviour in *Saccostrea cucullata*. Changes in vertical movement and horizontal swimming behaviour near the bottom were studied making use of computer-video motion analyses. This study showed for the first time a three way chemical communication between oyster larvae and adult conspecifics of *S. cucullata*. Adult oysters produce one or more chemical substances inducing oyster larvae to produce another (set of) chemical compound(s). This (those) chemical compound(s) induce(s) production of (a) certain metabolite(s) in adult oysters. This (those) metabolite(s) is (are) responsible for the inducement of settling behaviour in *S. cucullata* larvae. Our study gives a strong indication that (the) chemical compound(s) released by the adult oysters are two times the same but released in different amounts.

Introduction

Simpson and Beck (1965) described the reproduction of marine invertebrates. They stated that a majority of marine invertebrates reproduce sexually by producing gametes and by releasing them in the surrounding water. Here external fertilisation occurs. The embryos develop into larvae. They spend hours to months in the water column before metamorphosing into the juvenile form. Quite often the time spent in the water column is related to the lunar cycle. During this time larvae are often suspended and carried by ocean

currents, serving as dispersal agent for the species (Palmer and Strathmann, 1981; Levin, 1984). This way of dispersing the larvae is extremely important for sedentary species, such as barnacles, oysters, tubedwelling worms and bryozoans.

Benthic colonisation requires that larvae both settle and metamorphose. Settlement is a reversible behavioural process including the contact and the exploration of substrates by larvae before metamorphosis. Metamorphosis on the other hand is an irreversible developmental process mediating the biochemical, physiological, and morphological transformation of an individual between distinct life forms. Morse (1990) reviewed the compounds inducing settlement and metamorphosis. He came to the conclusion that for larvae of a given species both events are often induced by identical compounds.

Gregarious settlement of larvae often leads to aggregation of conspecific adults (Meadows and Campbell, 1972; Burke, 1986; Gotelli, 1990). For sessile or sedentary species reproducing sexually by spawning gametes into surrounding waters aggregation resulting from larval settlement can be critical. Once the gametes are released in the surrounding waters the hydrodynamic properties of turbulent flowing water tend to dilute and disperse them. This decreases the likelihood of fusion between eggs and sperm. The same problem occurs when the distance between spawning individuals of opposite sexes is increasing (Pennington, 1985; Grosberg, 1987; Denny and Shibata, 1989; Levitan, 1991). Gregarious settlement leading to aggregation among juvenile and adult conspecifics has a number of other advantages: increased protection from predation (Sebens, 1983; Keough, 1984), increased competitive ability (Buss, 1981), increased filter feeding efficiency (Hughes, 1978), and reduced juvenile and adult mortality (Knight-Jones, 1951; Highsmith, 1982; Young, 1983). Gregariousness is critical for many sessile and sedentary species. We can expect specific actions that promote the active selection of settlement sites by larvae.

There is a good possibility gregarious settlement occurs in response to chemical substances emitted by adult specimens of the species. A good overview of the existing literature on this topic is given by Crisp (1974), Burke (1986) and Pawlik and Hadfield (1990). A number of authors (Pawlik and Faulkner, 1986; Jensen and Morse, 1990) have made considerable progress in identifying the chemical substances laying the foundation of gregarious settlement by marine invertebrates. Up to now those chemical substances are not completely isolated or fully characterised. Cole and Knight-Jones (1939) were

the first to describe the gregarious settlement of oyster larvae. Since then there has been debate about the sources of the compounds inducing settlement. Three groups of researchers with different view points have emerged. The first group points to juvenile and adult oysters as the source of substances mediating the settlement of the larvae (Walne, 1966; Bayne, 1969; Hidu, 1969; Keck *et al.*, 1971; Veitch and Hidu, 1971, Hidu *et al.*, 1978). The second group thinks biofilms on oyster shell surfaces are the source of inducer molecules (Bonar *et al.*, 1986, 1990; Fitt *et al.,* 1990; Weiner *et al.*, 1989). A third group (Fitt *et al.*, 1989) stresses the role of ammonia as an inducer of larval settlement. Tamburri *et al.* (1992) were the first to study the effects of substances released by live adult oysters (*Crassostrea virginca*) and by biofilms within a single study. Their results showed that: (1) settlement inducers are produced both by oysters and biofilms, (2) larval settling behaviour in response to inducers from each source is essentially identical, and (3) the settlement inducers liberated by both sources have a molecular weight between 500 and 1000.

Although a topic of considerable research for the past 50 years the identity of inducers mediating larval settlement remains unsolved. Zimmer-Faust *et al.* (1994a, 1994b) examined the chemical identity of water-soluble compounds released by both adult conspecifics and bacteria biofilms influencing settlement of *Crassostrea virginica* larvae.

In this study we made use of a similar technique as Tamburri *et al.* (1992) to quantify larval responses of *Saccostrea cucullata* to waterborne substances released by each source. We showed that before metabolites, inducing settling behaviour, are released by adult *S. cucullata* specimens, a three way chemical communication between oyster larvae and adult oysters is necessary. This study does not give evidence chemical substances released by biofilms play a role in the inducement of settling behaviour.

Material and methods

Oysters

In April 1994, live oysters and empty oyster shells (called 'cultch') were collected from mangrove trees (*Rhizophora mucronata*) in a mangrove area adjacent to Gazi, a village approximately 50 km south of Mombasa (Kenya).

All oysters were collected at the same height above datum and had comparable lengths and widths. The oysters were transported out of the water to the laboratory of the Kenya Marine and Fisheries Research Institute. Transport did not exceed 3 hours. The animals were maintained in unfiltered, aerated, running sea water from Tudor Creek (temperature: 27 °C; salinity: 32‰; pH: 6.8) prior to use in experiments, and the animals were not fed any supplementary food. The oysters were kept in 'grazing chambers' (make Vitrost) simulating tidal cycling.

Larval cultures

Twenty eight days old larvae, raised from Gazi Bay oysters and spawned at the Kenya Marine and Fisheries Research Institute (Mombasa, Kenya), were used for all experiments. They were kept at 0.5 to 1.0 larvae.ml^{-1} in natural seawater at 24‰ salinity, pH 6.8, in a 30 °C incubater with a 12:12 dark:light cycle (light on 06.30 a.m.). Prior to use, the natural seawater was filtered over a 0.22 µm filter and autoclaved for 15 min at 150 °C and 15 psi. Cultures were aerated by air bubbled through Pasteur pipettes. The culture medium was changed daily to preclude the build up of pathogenic bacteria (Loosanoff and Davis, 1963). The oyster larvae were fed daily with a mixture of marine diatoms (*Chaetoceros sp.* and *Nitzschia sp.*) at ± 2.5 x 10^4 cells/ml.
Saccostrea cucullata larvae are typically ≥ 300 µm in length and have pigmented eye spots when competent to settle and metamorphose (Tack, 1997). Experiments started within 6 hours after 100% of the larvae had developed eyes, and experiments were run for 24 h thereafter.

Removal of biofilms

The method used to remove biofilms from the oyster shells was described by Tamburri *et al.* (1992). We focused on eliminating external biofilms because the microflora inside of oysters was numerically insignificant in comparison. The total numbers of bacteria in oyster tissues, on inner shell surfaces, and released into bath solutions during incubations of oysters were measured using epifluorescence microscopy. The amount of bacteria found was ≤ 0.4% of the total on the external shell surfaces [9.6 (± 0.3) x 10^6 compared with 3.5 (± 0.2) x 10^4]. The internal microflora and/or the internal bacteria are

therefore not further discussed is this article.
Microflora were removed from the outer surfaces of cultch and live oyster shells by first vigorously scrubbing with a soft plastic bristle brush to manually dislodge most of the biofilm material. The oysters and cultch then were bathed in 2.5% sodium hypochlorite (NaOCl) for 5 min, which oxidised and further removed micro-organisms from the shell surfaces. After those 5 min, each item was removed from the NaOCl solution and rinsed ten times with 200 ml of sterile artificial seawater for 5 min, followed by a second set of ten rinses. Living oysters, with shells treated mechanically and chemically, resumed pumping within minutes of being returned to seawater medium.
As a precaution, we performed chemical assays to test whether the final rinse waters were free of NaOCl. Rinse waters were analysed colorimetrically for total chlorine according to the DPD method with N,N-diethyl-p-phenylenediamine reagent. A spectrophotometer was used to measure peak absorbence at 445 nm. Because final rinse waters and artificial sea water both had identical chlorine levels (=2.2 µg/l), we concluded that the rinses removed all traces of NaOCl.
Oysters with removed external biofilms served principally as a source of oyster metabolites. Biofilms growing either on live oysters that were clamped shut, or on aged shell cultch, served as a source of biofilm metabolites. The oysters were shut with sterilised C-clamps.

Preparation of solutions

The methods used in preparing solutions are shown schematically in Tables 1 to 10. During the preparation of the different solutions all lab wares were sterilised before use by rinsing with 70% isopropyl alcohol.
All oysters used in test and control solutions were placed, 24 hours before use, in sterile artificial seawater with the same pH (6.8), the same salinity (24‰) and the same temperature (30 °C) as the larval culture medium.
For the preparation of the test solutions OLBS1, OLS1, LBS1 and LS1 five live *S. cucullata* oysters and 5000 oyster larvae were placed in 5 l sterile artificial seawater for a period of 4 hours. The way the oysters were treated is shown in Table 1. After the four hour bath, we removed the oysters and filtered the solutions to 0.22 µm. Filtered solutions were frozen at -87 °C until used in experiments.
Test solutions in Table 2 (OBS, OS, BS1 and S1) were made by bathing five

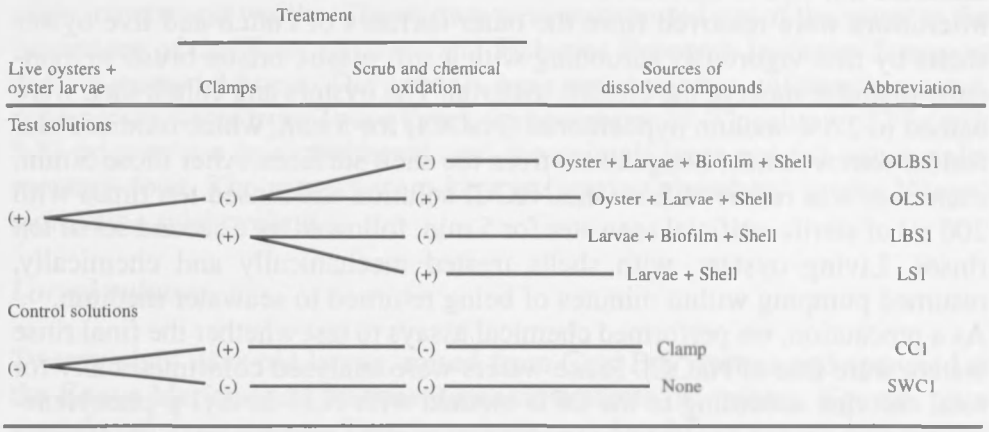

Table 1. Schematic representation of methods used to treat live oysters during the preparation of test and control solutions for experiment 1. (+) indicates the presence of oysters or use of treatment. (-) indicates the absence of oysters or omission of treatment.

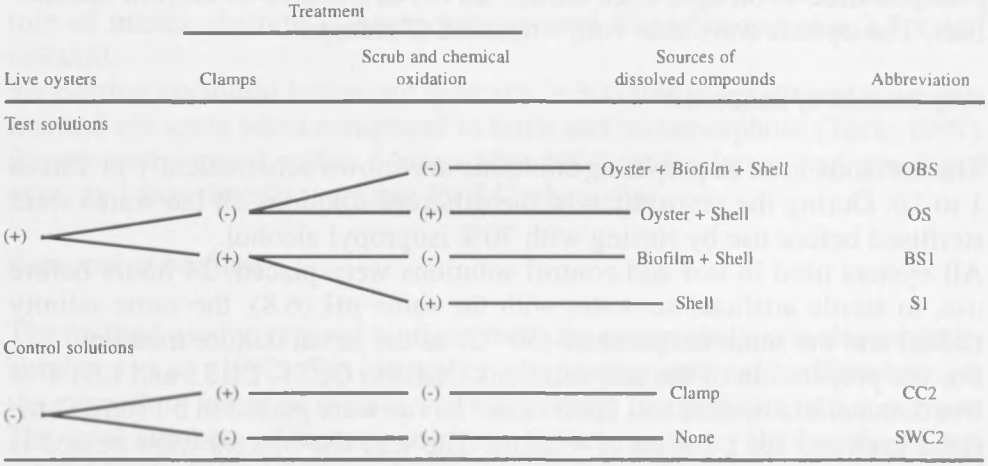

Table 2. Schematic representation of methods used to treat live oysters during the preparation of test and control solutions for experiment 2. (+) indicates the presence of oysters or use of treatment. (-) indicates the absence of oysters or omission of treatment.

oysters (treatment shown in Table 2), for 4 hours, in 5 l sterile artificial seawater. The solutions were filtered to 0.22 µm and frozen at -87 °C until used in experiments.
Test solutions in Table 3 (BS2 and S2) were made by bathing five empty oyster shells, for 4 hours, in 5 l sterile artificial seawater. Table 3 shows the way the empty shells were treated. After the four hour bath the solutions were filtered to 0.22 µm and frozen at -87 °C until used in experiments.
The test solution in Table 4 was made by bathing 5000 oyster larvae, for 4 hours, in 5 l sterile artificial seawater. After 4 hours the solution was filtered to 0.22 µm and used in the preparation of solutions OLBS2, OLS2, LBS2 and LS2 (Table 5). For each of those solution 5 oysters were brought into 5

	Treatment		
Shell cultch	Scrub and chemical oxidation	Sources of dissolved compounds	Abbreviation
Test solutions			
(+)	(-)	Biofilm + Shell	BS2
	(+)	Shell	S2
Control solutions			
(-)	(-)	None	SWC3

Table 3. Schematic representation of methods used to treat oyster cultch during the preparation of test and control solutions for experiment 3. (+) indicates the presence of cultch or use of treatment. (-) indicates the absence of cultch or omission of treatment.

Oyster larvae	Sources of dissolved compounds	Abbreviation
Test solutions		
(+)	Larvae	L

Table 4. Schematic representation of the preparation of test solution L used to prepare solutions for experiment 4. (+) indicates the presence of oyster larvae.

L + live oysters	Treatment: Clamps	Treatment: Scrub and chemical oxidation	Sources of dissolved compounds	Abbreviation
Test solutions				
(+)	(-)	(-)	Oyster + Larvae + Biofilm + Shell	OLBS2
		(+)	Oyster + Larvae + Shell	OLS2
	(+)	(-)	Larvae + Biofilm + Shell	LBS2
		(+)	Larvae + Shell	LS2
Control solutions				
(-)	(+)	(-)	Clamp	CC3
	(-)	(-)	None	SWC4

Table 5. Schematic representation of methods used to treat live oysters during the preparation of test and control solutions for experiment 4. (+) indicates the presence of oysters or use of treatment. (-) indicates the absence of oysters or omission of treatment. All solutions were made in L solution.

l of solution L for a period of 4 hours. After 4 hours oysters were taken out of the L solution and the new solutions were filtered to 0.22 µm and frozen at -87 °C until used in experiments.
The test solution OLS3 in Table 6 was made by bathing 5 *S. cucullata* oysters, for 4 hours, in 5 l solution OS. After 4 hours the solution was filtered to 0.22 µm and used for the preparation of solutions OLBS3, OLS4, LBS3 and LS3. For each of those solution 5 oysters (treatment described in Table 7) were brought into 5 l of solution OLS3 for a period of 4 hours. After 4 hours oysters were taken out of the solution and the new created solutions were filtered to 0.22 µm and frozen at -87 °C until used in experiments.
The oysters used to prepare the OLBS3 solution were used for dissection. The mantle lobes, the gills, the palps, the stomach, the excretory system and the gonads of the five oysters were crushed and mixed with 0.5 l sterile artificial seawater, giving us the solutions as mentioned in Table 8.
The OLS4 solution (Table 7) was diluted with sterile artificial seawater to 0.001x, 0.005x, 0.01x, 0.02x, 0.03x, 0.04x and 0.05x its original concentration. To each of the dilutions (5 l) 5000 oyster larvae were added. After 4 hours those new solutions (Table 9) were filtered to 0.22 µm. To each solu-

OS + Oyster larvae	Sources of dissolved compounds	Abbreviation
Test solutions		
(+)	Larvae	OLS3

Table 6. Schematic representation of the preparation of test solution OLS3 used to prepare solutions for experiment 5. (+) indicates the presence of oyster larvae and OS solution.

	Treatment			
OLS3 + live oysters	Clamps	Scrub and chemical oxidation	Sources of dissolved compounds	Abbreviation
Test solutions				
(+)	(-)	(-)	Oyster + Larvae + Biofilm + Shell	OLBS3
		(+)	Oyster + Larvae + Shell	OLS4
	(+)	(-)	Larvae + Biofilm + Shell	LBS3
		(+)	Larvae + Shell	LS3
Control solutions				
(-)	(+)	(-)	Clamp	CC4
	(-)	(-)	None	SWC5

Table 7. Schematic representation of methods used to treat live oysters during the preparation of test and control solutions for experiment 5. (+) indicates the presence of oysters or use of treatment. (-) indicates the absence of oysters or omission of treatment. All solutions were made in OLS3 solution.

tion 5 *S. cucullata* oysters were added. After the four hour bath, we removed the oyster. The solutions were filtered to 0.22 µm and frozen at -87 °C until used in experiments.

Larval behaviour analysis

Four to five hours before experiments were carried out, the larvae were carefully filtered from the culture media, rinsed and placed at 1 larvae.ml^{-1} in a

Test solutions	Abbreviation
Treated* mantle lobes	TML
Treated* gills	TGI
Treated* palps	TP
Treated* stomach	TS
Treated* excretory system	TE
Treated* gonads	TG

Table 8. Schematic representation of the preparation of test solutions TML, TGI, TP, TS, TE and TG for experiment 6.

Test solutions	Abbreviation		Abbreviation
0.001 OLS4 + larvae	OL1	+ oyster	OLO1
0.005 OLS4 + larvae	OL2	+ oyster	OLO2
0.010 OLS4 + larvae	OL3	+ oyster	OLO3
0.020 OLS4 + larvae	OL4	+ oyster	OLO4
0.030 OLS4 + larvae	OL5	+ oyster	OLO5
0.040 OLS4 + larvae	OL6	+ oyster	OLO6
0.050 OLS4 + larvae	OL7	+ oyster	OLO7

Table 9. Schematic representation of the preparation of test solutions OLO1, OLO2, OLO3, OLO4, OLO5, OLO6 and OLO7 for experiment 7.

separate container of sterile artificial seawater with the same temperature, salinity and pH as the seawater in which the oyster larvae were cultured. Making use of this procedure we were able to remove the oyster larvae from their micro-algal food. This food source influences the locomotory behaviour (Tamburri *et al.*, 1992) of the oyster larvae.

From now on all experiments were carried out in a dark, infrared (> 820 nm) illuminated chamber at 28 °C. Each experiment exists of a number of sub experiments. For each sub experiment 500 (vertical migration) or 100 (horizontal migration) oyster larvae were transferred, in 5 ml artificial seawater, into a Plexiglas® microcosm (3 cm long x 3 cm wide x 4 cm high) containing 25 ml of a test or control solution at a temperature of 30 °C. Those solutions were aerated for 5 seconds, then held still until the fluid came to rest. The larval movements were now recorded on video tape. The chamber was illuminated by infrared light oriented 90° to the axis of the video. The use of infrared light was required because oyster larvae exhibit phototactic responses to visible wavelengths (Smith and Chanley, 1975). The larvae were taped with a Sony infrared-sensitive video camera (Model HVM-200) placed on a Wild-Heerbrugg binocular with a magnification of 120 (10 x 12). Video records were made with a Panasonic NV-FS200 HQ on magnetic tape.

We monitored the oyster larvae during the initial 2 min of exposure. During the first two minutes we either measured a 100% settlement or no settlement at all. During the experiments we recorded larval movements in two directions: (1) larvae swimming vertically in the water column, and (2) larvae swimming horizontally near the bottom. Individual larvae were used only once in the experiments.

To study the vertical swimming behaviour of the oyster larvae we mounted the video camera at the side of the microcosm. The centre of the objective was mounted 1.5 cm (40 to 50 body lengths) above the bottom of the microcosm. The size of the viewing field was 12.2 mm x 12.2 mm, with a depth of 4.8 mm. Making use of motion analysis we counted the larvae moving vertically upward and downward in the viewing field. The number of larvae swimming downward was subtracted from the number of larvae swimming upward through the plane per unit time. This gives a quantitative measure of net vertical flux. A negative value means net downward movement, while a positive value means a net upward movement. Ten replicate trials were run for each solution.

To observe the horizontal swimming behaviour of the *S. cucullata* larvae the camera was mounted beneath the microcosm to view the larvae as they swam in a horizontal plane (12.2 mm x 12.2 mm) at a distance ≤ 4.8 mm (± 16 body lengths) above the bottom. For each solution 25 horizontal swimming paths were analysed making use of motion analysis software.

To analyse larval behaviour as a response to the different test and control

solutions we developed a computer-video motion analysis software in the C^{++} programming language. The video images were transferred from magnetic tape to hard disc making use of a miroMOTION DC 20 video card in combination with an Apple Power Macintosh 7600/120. The video motion analysis hardware was set to 25 frames/s. The motion analysis software was able to determine the swimming speed, the net-to-gross displacement ratio (NGDR), the rate of change in direction (RCD), and path duration, for each swimming path. The RCD is the angle turned per unit time, measured in degree/s. The NGDR is the ratio of the linear distance between the starting and ending points (net distance) and the total distance traversed by the path (gross distance). The NGDR measures the tendency of paths to be circular or twisted and reaches a minimum value of zero for looping or circular paths that have their origin and endpoint at the same spatial co-ordinates. An NGDR of 1.0 defines a completely straight path. Data for all paths were pooled across trials for each test or control treatment.

The effects of the different treatments on the vertical swimming behaviour were studied by a counting procedure. Under normal circumstances we would make use of a $\chi 2$ test statistics to study differences between the samples used in the experiments. However, in a majority of the experiments we expect no vertical movement and a measurement (counts) close to zero. Cochran (1954) recommends that for χ^2 tests with df larger than 1, fewer than 20% of the cells should have an expected frequency of less than 5 and no cell should have an expected frequency of less than 1. This is certainly not the case in our experiments. In this situation there is no clear alternative to the $\chi 2$ test.

ANOVA assumptions were tested on the variables measured for the different solutions. For each test we came to the same conclusions: (1) sampling of individuals was done at random; (2) performing a runs test the number of runs was not significantly different ($p < 0.05$) from expectation; (3) a F_{max}-test showed the variances of all samples were homogeneous; (4) we computed the χ^2-test and Kolmogorov-Smirnov single-sample test to evaluate the fit of the observed data to the normal distribution. Neither showed a significant difference ($p < 0.05$) between the observed data and the normal distribution; and (5) Tukey's test was not significant so we do not have evidence of non-additivity.

Since our data meet the assumptions for ANOVA testing anyhow, we preferred to compare the effects of the different treatments using Newman-

Keuls testing. An experiment-wise error rate of p=0.05 was used to determine when effects were significantly different. The choice of a Newman-Keuls test, a post-hoc test, was made because settlement hypothesis were only made after the experiments.
Newman-Keuls testing was also used to study the effect of the different treatments on the vertical behaviour. We preferred the use of nonparametric statistics because of the relative small samples. However, Newman-Keuls was used because: (a) there is no general nonparametric statistical test in a k-sample case with interval data; (b) using a nonparametric test on the ordinal level of measurement would conceal too much valuable information present in the data; (c) the samples, though relatively small, meet all the assumptions to perform an ANOVA; and (d) hypothesis were made after performing the experiments.

Experiments

In a first experiment oyster larvae brought in the test and control solutions of Table 1 were analysed for vertical and horizontal swimming behaviour. In experiment 2,3,4,5,6 and 7 the oyster larvae were brought in respectively the test and control solutions of Table 2, 3, 5, 7, 8, and 9 and were analysed.

Results

Mean (± S.D.) vertical movements of oyster larvae, when exposed to test and control solutions, are shown in Figures 1 to 7. The oyster larvae brought in contact with waterborne compounds in the OLBS1, OLS1, OLBS3, OLS4, TG, OLO3, OLO4, OLO5, OLO6 and OLO7 solutions responded in a manner indicative of settling behaviour. Oyster larvae exposed to the above mentioned solutions moved rapidly (within the first minute) downwards in the water column as shown in Figures 1, 5, 6 and 7.
There was no net vertical movement of oyster larvae when exposed to seawater controls (SWC1 to SWC5). Larval responses to substances released by shells and biofilms (BS1, BS2, LBS1, LBS2 and LBS3), shells alone (S1, S2, LS1, LS2 and LS3) and clamps alone (CC1 to CC4) were not significantly different from the vertical movement seen in the seawater controls. Oyster larvae did not respond to chemical compounds in OBS, OS, OLBS2

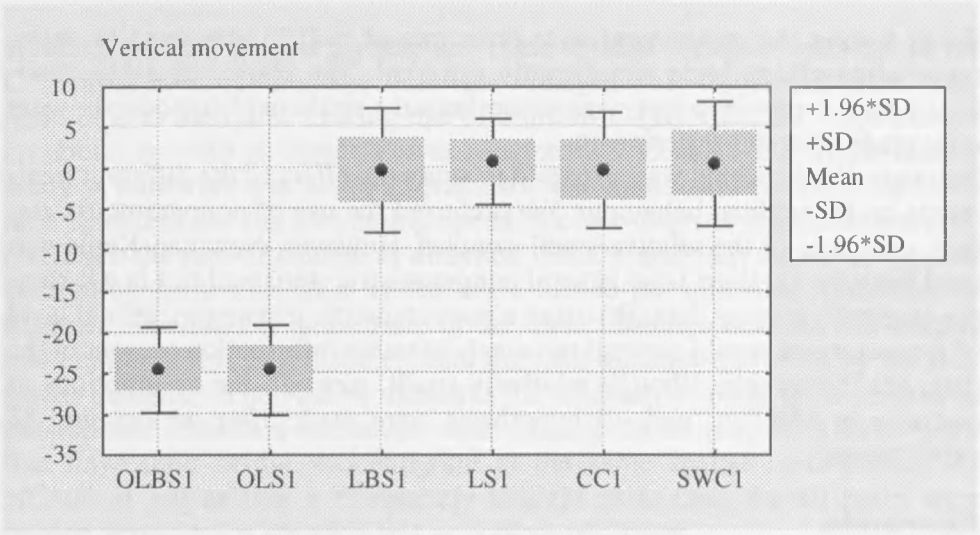

Figure 1. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. OLBS1 = OLS1 ≠ LBS1 = LS1 = CC1 = SWC1 (Newman-Keuls test: $F = 151.7$; $df = 5$; $p < 0.001$). The equal sign (=) indicates vertical fluxes that are not significant different. The Unequal sign (≠) indicates vertical fluxes that are significant different.

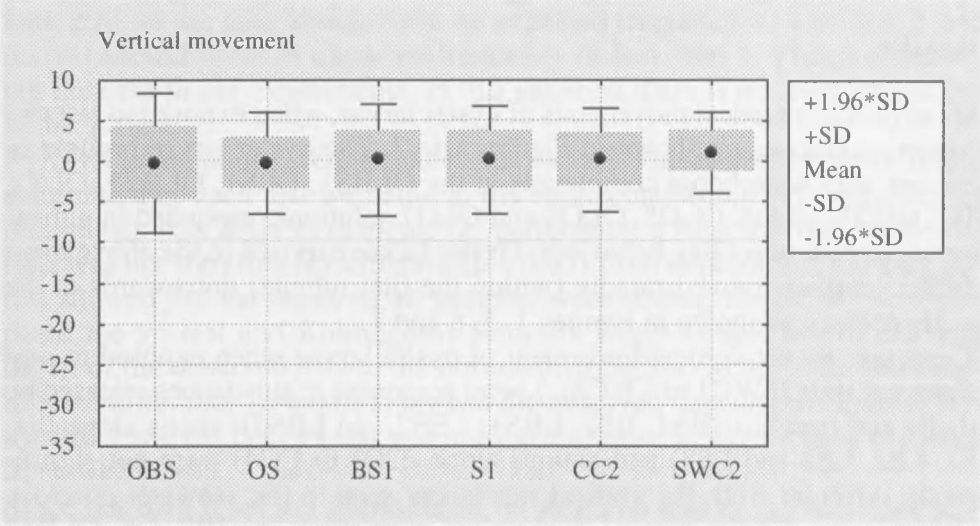

Figure 2. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. OBS = OS = BS1 = S1 = CC2 = SWC2 (Newman-Keuls test: $F = 0.22$; $df = 5$; $p > 0.95$). The equal sign (=) indicates vertical fluxes that are not significant different.

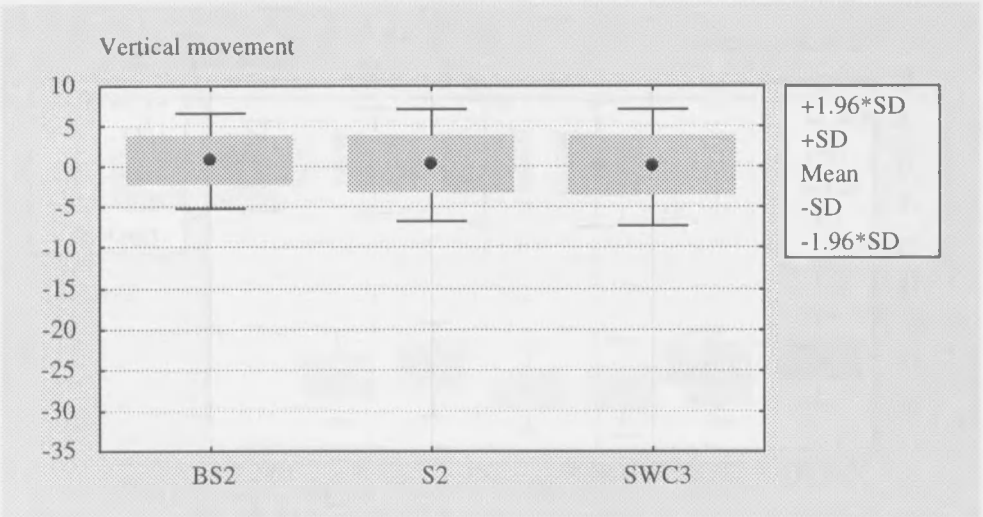

Figure 3. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. BS2 = S2 = SWC3 (Newman-Keuls test: F = 0.15; df = 2; p > 0.86). The equal sign (=) indicates vertical fluxes that are not significant different.

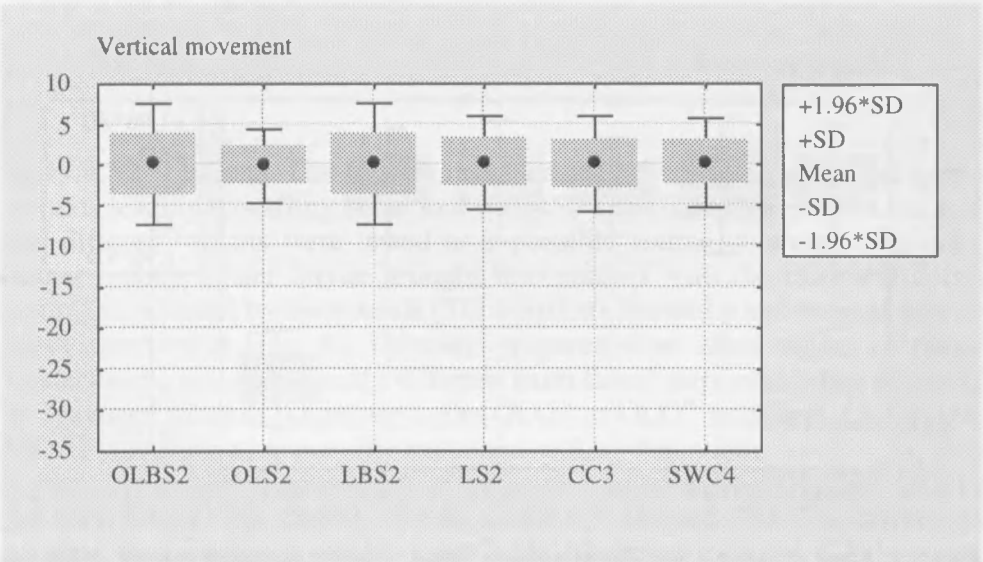

Figure 4. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. OLBS2 = OLS2 = LBS2 = LS2 = CC3 = SWC4 (Newman-Keuls test: F = 0.023; df = 5; p > 0.99). The equal sign (=) indicates vertical fluxes that are not significant different.

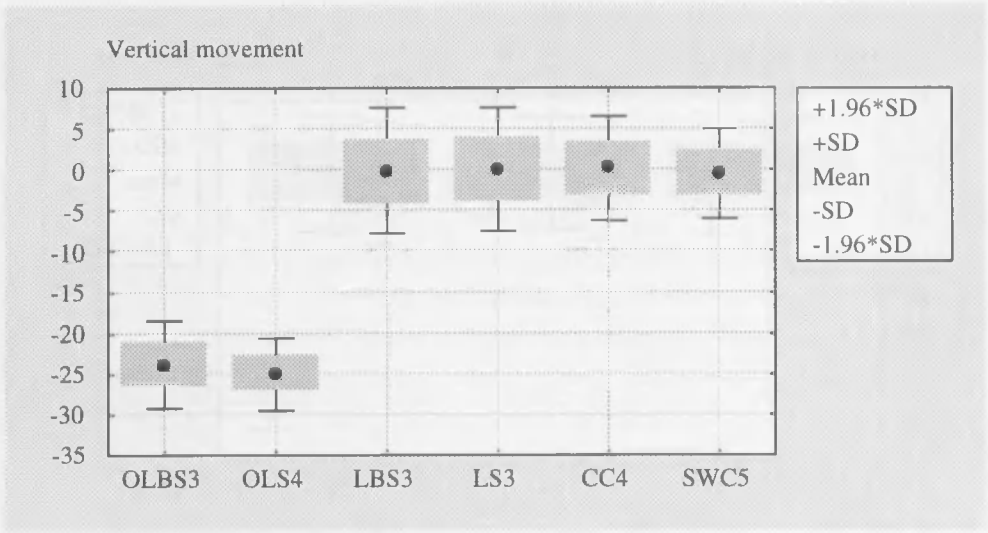

Figure 5. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. OLBS3 = OLS4 ≠ LBS3 = LS3 = CC4 = SWC5 (Newman-Keuls test: $F = 153.6$; $df = 5$; $p < 0.001$). The equal sign (=) indicates vertical fluxes that are not significant different. The Unequal sign (≠) indicates vertical fluxes that are significant different.

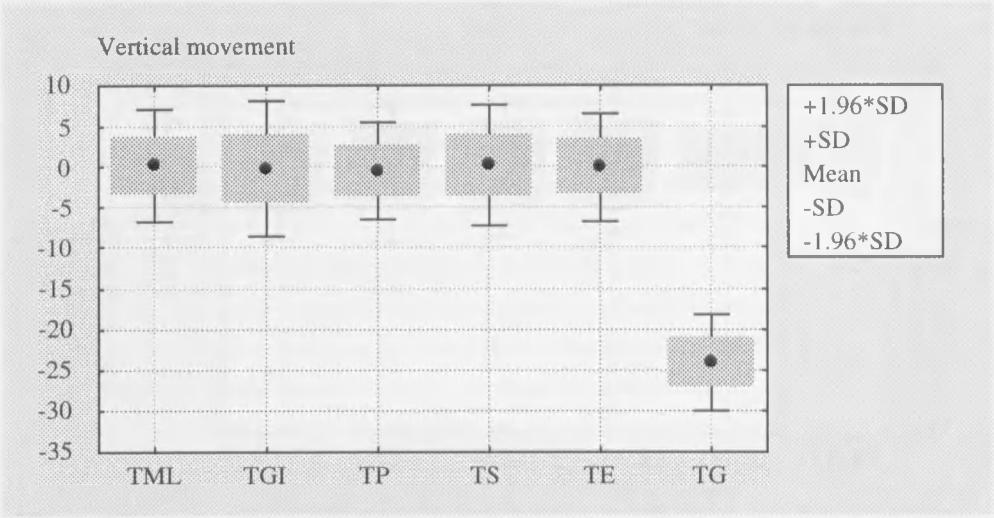

Figure 6. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. TML = TGI = TP = TS = TE ≠ TG (Newman-Keuls test: $F = 76.6$; $df = 5$; $p < 0.001$). The equal sign (=) indicates vertical fluxes that are not significant different. The Unequal sign (≠) indicates vertical fluxes that are significant different.

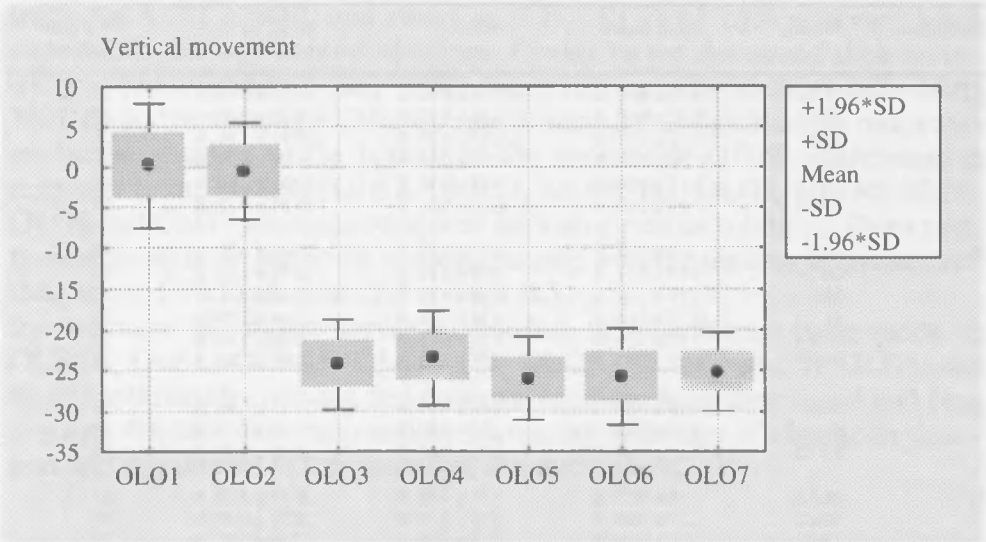

Figure 7. Mean (±SD; ± 1.96 SD) net vertical fluxes of oyster larvae swimming in the water column of the microcosm (n = 10). Negative results indicate a downward movement, positive results indicate an upward movement. OLO1 = OLO2 = OLO3 = OLO4 = OLO5 ≠ OLO6 = OLO7 (Newman-Keuls test: $F = 161.8$; $df = 6$; $p < 0.001$). The equal sign (=) indicates vertical fluxes that are not significant different. The Unequal sign (≠) indicates vertical fluxes that are significant different.

and OLS2 solutions. The oysters in experiment 5 releasing chemical compounds leading to settling behaviour (OLBS3 and OLS4) were dissected and the different organs were tested as a possible source of waterborne substances. Only oyster larvae brought into contact with the chemical compound(s) released by the gonads (TG solution) showed a net vertical movement downwards (Fig. 6). Solutions prepared from other organs of those oysters were not significantly different from larval responses when exposed to seawater controls. Opposite to the OLO3 to OLO7 solutions, OLO1 and OLO2 solutions were not able to induce settling behaviour.

Oyster larvae also responded to waterborne compounds in OLBS1, OLS1, OLBS3, OLS4, TG, OLO3, OLO4, OLO5, OLO6 and OLO7 solutions by altering their swimming behaviour. Table 10 shows mean (± S.D.) speed,

Experiment	Solution	Speed (mm/s)	NGDR	RCD(°/s)	# of paths analysed
I	OLBS1	0.43 ± 0.02 A	0.16 ± 0.02 A	74.55 ± 6.87 A	25
	OLS1	0.42 ± 0.02 A	0.16 ± 0.02 A	72.16 ± 7.66 A	25
	LBS1	1.18 ± 0.07 B	0.74 ± 0.09 B	21.92 ± 2.36 B	25
	LS1	1.19 ± 0.06 B	0.74 ± 0.11 B	21.99 ± 1.91 B	25
	CC1	1.17 ± 0.08 B	0.72 ± 0.12 B	22.21 ± 2.44 B	25
	SWC1	1.15 ± 0.06 B	0.75 ± 0.11 B	21.35 ± 2.17 B	25
II	OBS	1.18 ± 0.07 B	0.73 ± 0.10 B	21.41 ± 2.28 B	25
	OS	1.15 ± 0.07 B	0.74 ± 0.10 B	22.78 ± 2.35 B	25
	BS1	1.16 ± 0.09 B	0.74 ± 0.11 B	21.64 ± 2.11 B	25
	S1	1.17 ± 0.06 B	0.74 ± 0.09 B	22.44 ± 2.20 B	25
	CC2	1.17 ± 0.09 B	0.72 ± 0.11 B	21.80 ± 2.14 B	25
	SWC2	1.18 ± 0.07 B	0.77 ± 0.09 B	22.02 ± 1.66 B	25
III	BS2	1.18 ± 0.08 B	0.69 ± 0.10 B	21.13 ± 2.06 B	25
	S2	1.15 ± 0.08 B	0.75 ± 0.10 B	22.00 ± 2.19 B	25
	SWC3	1.18 ± 0.07 B	0.72 ± 0.10 B	22.15 ± 1.89 B	25
IV	OLBS2	1.18 ± 0.07 B	0.71 ± 0.08 B	21.56 ± 2.32 B	25
	OLS2	1.17 ± 0.08 B	0.76 ± 0.10 B	22.38 ± 2.20 B	25
	LBS2	1.17 ± 0.07 B	0.74 ± 0.10 B	22.18 ± 2.07 B	25
	LS2	1.17 ± 0.08 B	0.74 ± 0.09 B	22.04 ± 2.12 B	25
	CC3	1.17 ± 0.08 B	0.78 ± 0.10 B	22.10 ± 2.12 B	25
	SWC4	1.16 ± 0.08 B	0.77 ± 0.11 B	22.05 ± 2.22 B	25
V	OLBS3	0.41 ± 0.02 A	0.17 ± 0.02 A	76.36 ± 6.30 A	25
	OLS4	0.42 ± 0.03 A	0.16 ± 0.02 A	75.34 ± 7.11 A	25
	LBS3	1.17 ± 0.07 B	0.72 ± 0.10 B	22.27 ± 2.37 B	25
	LS3	1.16 ± 0.07 B	0.75 ± 0.09 B	21.12 ± 2.01 B	25
	CC4	1.18 ± 0.07 B	0.76 ± 0.11 B	22.43 ± 2.20 B	25
	SWC5	1.14 ± 0.07 B	0.75 ± 0.11 B	21.94 ± 2.39 B	25
VI	TML	1.16 ± 0.07 B	0.72 ± 0.08 B	21.95 ± 2.30 B	25
	TGI	1.17 ± 0.07 B	0.74 ± 0.10 B	21.94 ± 2.23 B	25
	TP	1.17 ± 0.07 B	0.71 ± 0.09 B	21.89 ± 2.18 B	25
	TS	1.17 ± 0.06 B	0.75 ± 0.09 B	22.59 ± 2.21 B	25
	TE	1.17 ± 0.09 B	0.75 ± 0.09 B	22.47 ± 2.27 B	25
	TG	0.42 ± 0.03 A	0.16 ± 0.02 A	74.68 ± 6.91 A	25
VII	OLO1	1.19 ± 0.08 B	0.72 ± 0.10 B	22.14 ± 2.17 B	25
	OLO2	1.20 ± 0.07 B	0.73 ± 0.11 B	22.10 ± 2.25 B	25
	OLO3	0.42 ± 0.02 A	0.17 ± 0.02 A	73.19 + 6.96 A	25
	OLO4	0.41 ± 0.02 A	0.16 ± 0.02 A	71.46 ± 7.73 A	25
	OLO5	0.41 ± 0.03 A	0.15 ± 0.02 A	72.38 ± 7.34 A	25
	OLO6	0.42 ± 0.03 A	0.16 ± 0.02 A	73.91 ± 7.90 A	25
	OLO7	0.42 ± 0.03 A	0.17 ± 0.02 A	72.98 ± 7.31 A	25

Table 10. Mean (±SD) swimming speed, mean (±SD) net-to-gross displacement ratio (NGDR) and mean (±SD) rate of change in direction (RCD) of larvae swimming near the bottom in test and control solutions. Capital letters designate swimming speeds (Newman-Keuls test: $F = 633.21$; $df = 39$; $p < 0.0001$), net-to-gross displacement ratios speeds (Newman-Keuls test: $F = 208.57$; $df = 39$; $p < 0.0001$) and changes in direction speeds (Newman-Keuls test: $F = 774.11$; $df = 39$; $p < 0.0001$), that are not significant different.

mean (± S.D.) NGDR and mean (± S.D.) RCD of oyster larvae, when exposed to test and control solutions. Oyster larvae decreased their swimming speeds, modified their path trajectories as expressed by decreasing NGDRs and increasing RCDs. We also observed that significantly more oyster larvae attached to the bottom of the microcosm (100% settlement) in response to the OLBS1, OLS1, OLBS3, OLS4, TG, OLO3, OLO4, OLO5, OLO6 and OLO7 solutions compared with all the other solutions. Oyster settlement in all other solutions was comparable with the number of oysters settling in the SWC solutions (2,3 ± 0.8 S.D.).
In summary all oyster larvae responded to waterborne compounds in OLBS1, OLS1, OLBS3, OLS4, TG, OLO3, OLO4, OLO5, OLO6 and OLO7 solutions by moving fast downwards, decreasing their speed and Net-to-gross displacement ratio and by increasing their rate of change in direction and attachment to the bottom of the microcosm.

Discussion

In 1939, Cole and Knight-Jones were the first to describe the gregarious settlement of oyster larvae. Since that moment several groups of view concerning sources of chemical inducers have emerged. A first group (Walne, 1966; Bayne, 1969; Hidu, 1969; Keck *et al.*, 1971; Veitch and Hidu, 1971, Hidu *et al.*, 1978) reports juvenile and adult oysters as the source of those chemical compounds. A second group (Bonar *et al.*, 1986, 1990; Weiner *et al.*, 1989) points towards biofilms as the main source of those waterborne compounds. A third group (Fitt *et al.*, 1989) stresses the role of ammonia as an inducer of oyster larvae settlement.
One of the main problems to compare the results of those groups are the enormous differences in experimental approach. It were Tamburri *et al.* (1992) who compared both adult oysters (*Crassostrea virginica*) and bacterial sources in the same experimental approach. They came to the conclusion that both sources produce waterborne substances rapidly provoking larval settlement.
Fitt *et al.* (1989) showed ammonia to be an inducer of settling behaviour of veligers in the genus *Crassostrea.* Eyed veligers exposed to oyster conditioned seawater responded only to seawater containing >100 µM ammonia/ammonium. This suggests ammonia is a natural cue. Natural levels of

ammonia in seawater are typically low. Increased concentrations of ammonia/ammonium occur in proximity to the substrate, especially when large aggregates of adult oysters form an oyster bed on top of the substrate. This is possible because ammonium is heavier than seawater. The typical growth of *S. cucullata* on stilt roots and stems of mangrove trees prevents the accumulation of ammonia/ammonium to sufficient high levels to act as a settlement inducer.

In this study we made use of an analogue procedure as Tamburri *et al.* (1992). This makes it possible to compare our results more easily. We made use of computer-video motion analysis to non-invasively track the path made by individual larvae as they moved vertically and horizontally through the water column. Tamburri *et al.* (1992) already showed that this kind of technology has numerous advantages: (1) experimental chambers having large volumes (e.g. 30-5000 ml: Zimmer-Faust, 1990; Weissburg and Zimmer-Faust, 1991) can be used, which do not impede locomotory behaviour, (2) locomotory paths can be tracked for many individual larvae simultaneously, and (3) data can be collected rapidly and in sufficient quantities from larvae held at low densities (1 or $2.ml^{-1}$), thereby avoiding interactions between larvae and density dependent effects.

Essential in our experiment was the isolation of oyster metabolites from biofilm metabolites. This was done by minimising the amount of biofilm organisms on the outer shell surfaces of a number of living adult oyster shells. We were only concerned with the removal of external biofilms for two reasons: (1) the number of bacteria in oyster tissues and on internal shell surfaces were numerically insignificant (≤0.4%) compared with the total on external shell surfaces; (2) we consider the internal bacteria as an inseparable part of the oyster itself. The method used in this study removed almost 99% of all bacteria of the external biofilm. Tamburri *et al.* (1992) showed that the use of this method did not effect the metabolism of the oysters treated.

When adult oysters of *S. cucullata* (Gazi Bay) and oyster larvae were brought together in a microcosm there was almost immediate settlement of the oyster larvae. After removing the oyster and the oyster larvae, the water in the microcosm was filtered to 0.22 µm. When we brought new larvae in this test solution (OLBS1, experiment 1) those larvae also settled immediately. Clearly chemical compounds were produced inducing settlement of *S. cucullata* larvae. The OLBS1 solution has four possible sources of dissolved

compounds in the water: oyster, larvae, biofilm and shell. We can expect oyster larvae are not releasing a chemical compound with a straight effect (we will call this a first order inducer) on their settlement. In this case oyster larvae settlement would occur in all experiments. In the OLS1 solution the external biofilm of the oyster used was removed. Oyster larvae settled when brought in this solution without chemical substances produced by an external biofilm. This indicates that the biofilm is not producing a necessary compound to induce settlement. Oyster larvae brought in test solutions LBS1 and LS1 showed no settling behaviour indicating that neither the shell, nor the biofilm was producing first order chemical compounds inducing settling behaviour. However, the possibility remains biofilms have to be triggered themselves before producing such a compound. In the second experiment we tried to find out whether adult oysters were producing a chemical substance able to induce settlement. Oyster larvae added to the OBS, OS, BS1 and S1 solutions showed no intention to settle. This indicates adult oysters are not first order inducers. Experiment three confirms that the biofilm and/or the shell is not producing a first order chemical compound inducing settlement. The oyster shell is build of dead material, so it is not able to react to a first order inducer. This eliminates the shell as a possible source of chemical substances inducing settling behaviour.

After the first three experiments we can make the following conclusions: (1) oyster larvae are not acting as a first order inducer of settling behaviour; (2) the adult oyster of *S. cucullata* is not producing a waterborne compound inducing settling behaviour of *S. cucullata* larvae (first order inducement), unless there is a mechanism where the oyster itself is induced in doing so; (3) if the adult oyster is producing a chemical compound inducing settlement it has to be induced by chemical substances released by oyster larvae; (4) the biofilm is not a first or a second order inducer; (5) the biofilm can be a higher order inducer, only when as well the adult oyster as the oyster larvae are part of the inducement process; (6) the shell is not acting as a first order inducer and it has not the possibility to act as a higher order inducer.

Because of the limited number of actors involved we restricted our research to the interaction between adult oysters and oyster larvae. We first tested the hypothesis that the oyster larvae were producing a chemical compound stimulating the adult oysters to release another chemical compound triggering settling behaviour of the *S. cucullata* larvae (experiment 4). Oyster larvae brought in OLBS2, OLS2, LBS2 and LS2 solutions showed no indications

of settling behaviour. Those results indicate that our hypothesis of the adult oyster as a second order inducer is incorrect. Experiment 5 tests the possibility of a three steps communication between adult oyster and their oyster larvae. Oyster larvae were brought into artificial seawater with chemical compounds originating from adult oysters. After 4 hours the oyster larvae were filtered out of this solution (OS) creating a new solution (OLS3). In this solution we brought again adult *S. cucullata* oysters. After 4 hours the solution was filtered to 0.22 µm and oyster larvae were added. They settled almost immediately. This experiment shows a three way communication between adult *S. cucullata* specimens and oyster larvae of the same species. Adult oysters release a chemical compound inducing the production of another chemical compound by the oyster larvae. This chemical substance induces adult oysters to produce a chemical compound responsible for the inducement of settling behaviour of *S. cucullata* larvae. By dissecting the oysters used in experiment 5 we tried to reveal the organ responsible for the production of the chemical substance inducing settlement. We tested mantle lobes, gills, palps, stomach, excretory system and gonads. Oyster larvae responded positive to chemical substances released by the gonads of the oysters of experiment 5 (solution TG). This clearly indicates that the responsible settlement inducer is produced in the gonads. Experiment 7 had the goal to test whether the chemical compound influencing oyster larvae could be twice the same in other dilutions. Oyster larvae were added to seven dilutions of the TG solution, respectively diluted for 1000x, 200x, 100x, 50x, 20x and 10x. After 4 hours oyster larvae were filtered out of the water and adult oysters were added. After another 4 hours the adult oysters were taken out of the solution and oyster larvae were added. In those cases where the TG solution was diluted for 1000x and 200x no settling behaviour was reported. In dilutions of 100x and lower the original used TG solution oyster larvae settled almost immediately. This experiment (Fig. 7) supports the hypothesis that adult oysters are releasing certain chemical compounds in relative small concentrations. Those relative small concentrations trigger the oyster larvae to produce other chemical substances. They will trigger the adult oyster to produce the same chemical substances as it was producing before but in much larger quantities. Once the concentration of those chemical compounds in the water reaches a certain level they will induce the settling behaviour of the oyster larvae. It is off course possible that only one compound is acting as a triggering agent towards the oyster larvae and another towards the adult

oysters of *S. cucullata*. This hypothesis does not exclude the possibility of biofilms playing a certain role in the settling behaviour of *S. cucullata*. However, if they play a role, the interaction between biofilm, adult oysters and oyster larvae will probably be much more complicated than the interactions between oyster larvae and adult oysters as shown above.
Larvae swimming in the close neighbourhood of the bottom (≤ 16 body lengths) responded similarly to chemical substances released by adult oysters and biofilms. Larvae swim actively downwards to the bottoms of the microcosms, decrease their swimming speed and net-to-gross displacement ratio and increase their rate of turning. These changes in swimming behaviour are consistent with site-restricted search in zooplankton larvae (Buskey, 1984; Buskey and Stoecker, 1989) and larvae of fiddler crabs (Weissburg and Zimmer-Faust, 1991). Increased turning may facilitate 'search' for specific microsites. Turning causes larvae to loop back focusing locomotory activity within a small area (Koopman, 1980).

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References

Bayne, G. L. (1969). The gregarious behavior of the larvae of *Ostrea edulis* L. at settlement. J. Mar. Biol. Assoc. U.K. 49: 327-356

Bonar, D. B., Weiner, R. M., Colwell, R. R. (1986). Microbial-invertebrate interactions and potential for biotechnology. Microb. Ecol. 12: 101-110

Burke, R. D. (1986). Pheromones and gregarious settlement of marine invertebrate larvae. Bull. Mar. Sci. 39: 323-331

Buskey, E. J. (1984). Swimming patterns as an indicator of the roles of copepod sensory systems in the recognition of food. Mar. Biol. 79: 165-175

Buskey, E. J., Stoecker, D. K. (1989). Behavioral responses of the marine tintinnid *Favella sp.* to phytoplankton: influence of chemical, mechanical and photic stimuli. J. Exp. Mar. Biol. Ecol. 32: 1-16

Buss, L. W. (1981). Group living, competition, and the evolution of cooperation in a sessile invertebrate. Science 213: 1012-1014

Cochran, W. G. (1954). Some methods for strengthening the commen χ^2 tests. Biometrics 10: 417-451

Cole, J. A., Knight-Jones, E. W. (1939). Some observations and experiments in the setting behavior of *Ostrea edulis*. J. Cons. Int. Explor. Mer. 14: 86-105

Crisp, D. J. (1974). Factors influencing settlement of marine invertebrate larvae. In: Grant, P. T., Mackie, A. M. (Ed.) Chemoreception in Marine Organisms. Academic Press, London, p. 177-265

Denny, M. W., Shibata, M. F. (1989). Consequence of surf-zone turbulence for settlement and external fertilization. Am. Nat. 134: 859-889

Fitt, W. K., Coon, S. L., Walch, M., Weiner, R. M., Colwell, R. R., Bonar, D. B. (1990). Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. Mar. Biol. 106: 389-394

Fitt, W. K., Labare, M. P., Fuqua, W. C., Walch, M., Coon, S. T., Bonar, D. B., Colwell, R. R., Weiner, R. M. (1989). Factors influencing bacterial production of inducers of settlement behavior of larvae of the oyster *Crassostrea gigas*. Microb. Ecol. 17: 287-298

Gotelli, N. J. (1990). Stochastic models of gregarious larval settlement. Ophelia 32: 95-108

Grosberg, R. K. (1987). Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. Evolution 41: 372-384

Hidu, H. (1969). Gregarious setting in the American oyster, *Crassostrea virginica* Gmelin. Chesapeake Sci. 10: 85-92

Hidu, H., Valleau, W. G., Veitch, F. P. (1978). Gregarious setting in the European and American oysters-response to surface chemistry vs. waterborne pheromones. Proc. Nat. Shellfish. Assoc. 68: 11-16

Highsmith, R. C. (1982). Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. Ecology 63: 329-337

Hughes, R. N. (1978). Coloniality in *Vermetidea* (Gastropoda). In: Larwood, G., Rosen B. R. (Ed.) Biology and Systematics of Colonial Organisms. Academic Press, New York, p. 319-327

Jensen, R. A., Morse, D. E. (1990). Chemically induced metamorphosis of polychaete larvae in both the laboratory and the ocean environment. J. Chem. Ecol. 16: 911-930

Keck, R., Maurer, D., Kauer, J. C., Sheppard, W. A. (1971). Chemical stimulants affecting larval settlement in the American oyster. Proc. Nat. Shellfish. Assoc. 61: 24-28

Keough, M. J. (1984). Kin-recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* (L.). Evolution 38: 142-147

Knight-Jones, E. W. (1951). Gregariousness and some other aspects of the settling behavior of *Spirorbis*. J. Mar. Biol. Assoc. U.K. 30: 201-222

Koopman, B. O. (1980). Search and Screening. Pergamon Press, New York

Levin, L. A. (1984). Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbances. Ecology 65: 1185-1200

Levitan, D. R. (1991). Influence of body size and population density on fertilization success

and reproductive output in a free-spawning invertebrate. Biol. Bull. 181: 261-268
Loosanoff, V. L., Davis, J. C. (1963). Rearing of bivalve mollusks. In: Russell, F. S. (Ed.) Advances in Marine Biology. Academic Press, London, p. 1-136
Meadows, P. S., Campbell, J. I. (1972). Habitat selection by aquatic invertebrates. Adv. Mar. Biol. 10: 271-282
Morse, D. E. (1990). Recent progress in larval settlement and metamorphosis: closing the gaps between molecular biology and ecology. Bull. Mar. Sci. 46: 465-483
Palmer, A. R., Strathmann, R. R. (1981). Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. Oecologia 48: 308-318
Pawlik, J. R., Faulkner, D. J. (1986). Specific free fatty acids induce larval settlement and metamorphosis of reef-building tube worm *Phragmatopoma californica* (Fewkes). J. Exp. Mar. Biol. Ecol. 102: 301-310
Pawlik, J. T., Hadfield, M. G. (1990). A symposium on chemical factors that influence the settlement and metamorphosis of marine invertebrate larvae: introduction and prospective. Bull. Mar. Sci. 46: 450-454
Pennington, J. T. (1985). The ecology of fertilization of echinoid eggs: the consequence of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. 169: 417-430
Sebens, K. P. (1983). Larval and juvenile ecology of the temperate octocoral *Alcyonium siderium* Verrill II. Fecundity, survivorship, and juvenile growth. J. Exp. Mar. Biol. Ecol. 72: 263-285
Simpson, G. G., Beck, W. S. (1965). Reproduction: larvae. In: Simpson, G. G., Beck, W. S. (Ed.) Life: An Introduction to Biology. Harcourt, Brace and World, Inc., New York, p. 266-267
Smith, W. L., Chanley, M. H. (1975). Provisions of light. In: Smith, W. L., Chanley, M. H. (Ed.) Culture of Marine Invertebrate Animals. Plenum Press, New York, p. 33-36
Tack, J. F. (1997). Spawning behaviour and larval culture of *Saccostrea cucullata* (von Born, 1778). In: Behavioural aspects of the mangrove oyster *Saccostrea cucullata* (von Born, 1778) explaining its macro and micro distribution along the Kenyan coast. Unpublished Ph. D. thesis, Vrije Universiteit Brussel, Brussels, p. 157-166
Tamburri, M. N., Zimmer-Faust, R. K., Tamplin, M. L. (1992). Natural Sources and Properties of Chemical Inducers Mediating Settlement of Oyster Larvae: A Re-examination. Biol. Bull. 183: 327-338
Veitch, F. P., Hidu, H. (1971). Gregarious setting in the American oyster *Crassostrea virginica* Gmelin: I. Properties of a partially purified "setting factor". Chesapeake Sci. 12: 173-178
Walne, P. R. (1966). Experiments in large-scale culture of the oyster *Ostrea edulis*. Fish. Invest. Lond. Ser. 2 25: 1-53
Weiner, R. M., Walch, M., Labare, M. P., Bonar, D. B., Colwell, R. R. (1989). Effect of biofilms of the marine bacterium *Alteromonas colwelliana* (LST) on the set of the oyster *Crassostrea gigas* (Thunberg, 1793) and *C. virginica* (Gmelin, 1791). J. Shellfish Res. 8: 117-123
Weissburg, M. J., Zimmer-Faust, R. K. (1991). Ontogeny versus phylogeny in determining patterns of chemoreception: initial studies with fiddler crabs. Biol. Bull. 181: 205-215
Young, C. M. (1983). Functional significance of larval behavior in an intertidal population of the ascidian *Pyura haustor*. Am. Nat. 23: 1021
Zimmer-Faust, R. K. (1990). Settlement behavior of larvae is revealed using computer-

video motion analysis. Am. Zool. 30: 556
Zimmer-Faust, R. K., Tamburri, M. N. (1994a). Chemical identity and ecological implications of a waterborne, larval settlement cue. Limnol. Oceanogr. 39: 1075-1087
Zimmer-Faust, R. K., Tamburri, M. N. (1994b). Chemical identity of oyster larval settlement cues. J. Shellfish Res. 13: 282

Aggregations of adult *Saccostrea cucullata* (von Born, 1778) specimens influence settling behaviour and survival of their larvae

Abstract

Chemical communication between adult oysters of *Saccostrea cucullata* and their larvae leads to the production of a chemical inducer mediating settlement of oyster larvae. This study shows how such a settlement inducer released by adult oysters provokes gregarious settlement. Gregarious settlement of *S. cucullata* larvae leads to aggregation of adult conspecifics. Experiments show how an aggregation of adult *S. cucullata* specimens attracts significantly more oyster larvae compared with a single adult oyster or no oyster at all. Also the survival rate of oyster larvae settled in the immediate neighbourhood of such an aggregation is much higher than the survival rate of larvae settled close to a single adult oyster.

Introduction

Cole and Knight-Jones (1939) were the first to describe the gregarious settlement of oyster larvae. Oysters reproduce sexually. They produce gametes and release them in the surrounding water. Here external fertilisation occurs. Those larvae spend a certain time, often related to the lunar cycle, in the water column. During this period the oyster larvae are suspended and carried by ocean currents. Those currents serve as dispersal agents for the species. This way of dispersing the larvae is not only extremely important for oysters,

but also for numerous other benthic organisms (Palmer and Strathmann, 1981; Levin, 1984).

Gregarious settlement often leads to aggregation of adult conspecifics (Meadows and Campbell, 1972; Burke, 1986; Gotelli, 1990). This can be very important for sessile or sedentary species reproducing sexually. Once their gametes are released in the surrounding water they will be dispersed and diluted, decreasing chances on a successful fertilisation. This problem grows when the distance between male and female individuals increases (Pennington, 1985; Grosberg, 1987; Denny and Shibata, 1989; Levitan, 1991). Gregarious settlement, leading to aggregation of adult conspecifics, has numerous advantages: increased protection from predation (Sebens, 1983; Keough, 1984), increased competitive ability (Buss, 1981), increased filter feeding efficiency (Hughes, 1978), and reduced juvenile and adult mortality (Knight-Jones, 1951; Highsmith, 1982, Young, 1983). Because of the importance of gregarious settlement for several species we can expect specific actions to promote gregarious settlement of larvae. Crisp (1974), Burke (1986) and Pawlik and Hadfield (1990) show that there is a good possibility that gregarious settlement occurs in response to chemical compounds released by adult conspecifics of the species involved.

Since Cole and Knight-Jones (1939) described gregarious settlement there has been debate on the possible sources of chemical substances mediating settlement behaviour of oyster larvae. A first group (Walne, 1966; Bayne, 1969; Hidu, 1969; Keck *et al.*, 1971) points towards the adult oyster as the source of the settlement inducing compounds. A second, more recent group (Bonar *et al.*, 1986, Fitt *et al.*, 1990; Weiner *et al.*, 1989) means that the origins of those compounds are to be found in the biofilm growing on shell surfaces of adult specimens. A third group of authors (Fitt *et al.*, 1989) stresses the role of ammonia as an inducer of larval settlement. Comparison between those viewpoints is extremely difficult because of a total difference in experimental approach. Tamburri *et al.* (1992) studied the influence of waterborne compounds produced by adult *Crassostrea virginica* oysters and biofilms within a single study. They came to the conclusion that: (1) settlement inducers are produced both by oysters and biofilms, (2) larval settlement behaviour in response to inducers from each source is essentially identical, and (3) inducers from both sources are amplifying each other. Tack (1997a) showed a more complex situation in the case of *Saccostrea cucullata*. They separated live adult oysters and biofilms as source of possible waterborne chemical

inducers of settlement behaviour in *Saccostrea cucullata*. Changes in vertical movement and horizontal swimming behaviour near the bottom were studied making use of computer-video motion analyses. Their study showed for the first time a three way chemical communication between oyster larvae and adult oysters of *S. cucullata*. Adult oysters produce one or more chemical substances inducing oyster larvae to produce another (set of) chemical compound(s). This (those) chemical compound(s) induce(s) production of (a) certain metabolite(s) in adult oysters. This (those) metabolite(s) is (are) responsible for the inducement of settling behaviour in *S. cucullata* larvae. The study also gives a strong indication that (the) chemical compound(s) released by the adult oysters are two times the same but released in different amounts.
The study of Tack (1997a) was performed in a microcosm (3 cm long x 3 cm wide x 4 cm high). The present study shows the effectiveness of waterborne compounds inducing gregarious settlement in a larger microcosm (1 m wide x 3 m long x 0.3 m high) and in natural conditions. Gregarious settlement of *S. cucullata* larvae leads to aggregation of adult conspecifics. This study shows that the survival of oyster spat is related to the presence of such aggregations.

Material and methods

Oysters

S. cucullata is growing on the trunks and stilt roots of mangrove trees. In this study we will make use of oysters collected from mangrove trees and of oysters growing on mangrove trees without being removed.
The mangrove forest in Gazi Bay, approximately 50 km south of Mombasa (Kenya), is relatively small but still in a more or less natural condition. Within the eastern creek the Kenya Belgium Project in Marine Sciences is exploiting a small scaled oyster culture. Experiments in 'natural' conditions and experiments in 'culture' conditions were carried out in Gazi Bay between June and September 1994. Oysters used in laboratory experiments were collected from mangrove trees (*Rhizophora mucronata*) in Gazi Bay in July 1994. All oysters were collected at the same height above datum and had comparable lengths and widths. Those oysters used in laboratory conditions

were transported out of the water to a laboratory of the Kenya Marine and Fisheries Research Institute. Oysters were maintained in unfiltered, aerated, running sea water from Tudor Creek (temperature: 25 °C; salinity: 34‰; pH: 6.9) prior to use in experiments.

Larval cultures

Twenty eight days old larvae, raised from Gazi Bay oysters and spawned at the Kenya Marine and Fisheries Research Institute (Mombasa, Kenya), were used for all experiments. They were kept at 0.5 to 1.0 larvae /ml in natural seawater at 24‰ salinity, pH 6.8, in a 30 °C incubater with a 12:12 dark:light cycle (light on 06.30 a.m.). Prior to use, the natural seawater was filtered over a 0.22 µm filter and autoclaved for 15 min at 150 °C and 15 psi. Cultures were aerated by air bubbled through Pasteur pipettes. The culture medium was changed daily to preclude the build up of pathogenic bacteria (Loosanoff and Davis, 1963). The oyster larvae were fed daily with a mixture of marine diatoms (*Chaetoceros sp.* and *Nitzschia sp.*) at $\pm 2.5 \times 10^4$ cells.ml^{-1}.
Saccostrea cucullata larvae are typically ≥ 300 µm in length and have pigmented eye spots when competent to settle and metamorphose (Tack, 1997b). Experiments started within 6 hours after 100% of the larvae developed eyes, and experiments were run for 24 h thereafter.

Chemical inducer

Tack (1997a) described five methods to produce solutions where the chemical compound(s) inducing settlement behaviour of oyster larvae is (are) present. We made use of the most simple method described by them. We brought 50 *S. cucullata* specimens and 50,000 oyster larvae in 50 l sterile artificial seawater (temperature: 30 °C, salinity: 24‰ and pH: 6.9) for a period of 4 hours. After the four hour bath, we removed the oysters and filtered the solution to 0.22 µm, and froze it at -87 °C until used in experiments. This solution will be called the SI solution (settlement inducer).

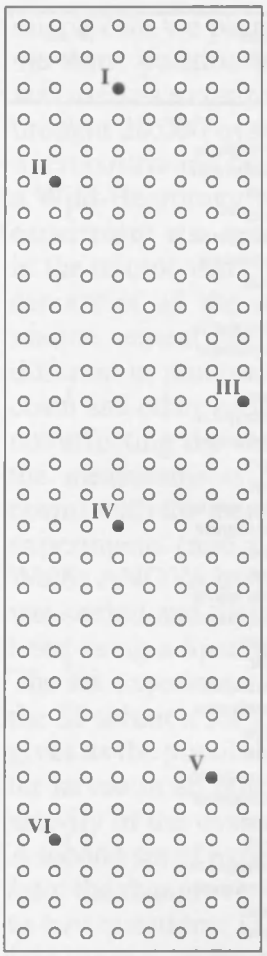

Figure 1. Microcosm: view from above. Black dots indicate sticks used as possible substrate for settlement of *S. cucullata* larvae. Latin numbers indicate at random positions at which the settlement inducer was released in subsequent experiments (see table 1).

Experiments

Tack (1997a) described a chemical inducer mediating settlement of *S. cucullata* larvae in a microcosm of 3 cm long, 3 cm wide and 4 cm high. In this study we are interested in the extrapolation of their results to the natural habitat of *S. cucullata*: the mangrove forest.

In a first set of experiments we made use of a much larger microcosm with a width of 1 m, a length of 3 m and a height of 0.4 m. Those experiments had two objectives: (1) to find the maximum distance from the source at which the settlement inducer is still active, and (2) to study the effect of the distance (the higher the distance, the higher the dilution of the settlement inducer) on the number of oyster larvae settling.

Table 1 shows an overview of the experimental setup of this first series of experiments. We made use of four microcosms, plastic containers with a width of 1m, a length of 3 m and a height of 0.4 m. Each microcosm was filled with natural seawater pumped out of Tudor Creek in front of the Kenya Marine and Fisheries Research Institute, Mombasa (Kenya). We adjusted the temperature to 30 °C, the salinity to 24‰ and the pH to 6.8 by adding 1.0 M NaOH. Those were the conditions under which the oyster larvae were reared. In each microcosm eight rows of wooden sticks were placed in such a way that all sticks were hanging on the corners of 10 x 10 cm squares (Fig. 1). Each stick was covered with a thin layer of marine cement, making it an excellent substrate for settlement. Marine cement is also used on the tiles used in the oyster cultures along the Kenyan coast.

In a first experiment we made use of live adult oysters as the source of settlement inducer. In each

Exp. N°	Source of settlement inducer	Place adult oyster in microcosm (see figure 1)	Variables studied	N° of replicas
I.A	live adult oyster	I	# spat; distance from spat to source of settlement inducer	3
II.A	live adult oyster	II	# spat; distance from spat to source of settlement inducer	3
III.A	live adult oyster	III	# spat; distance from spat to source of settlement inducer	3
IV.A	live adult oyster	IV	# spat; distance from spat to source of settlement inducer	3
V.A	live adult oyster	V	# spat; distance from spat to source of settlement inducer	3
VI.A	live adult oyster	VI	# spat; distance from spat to source of settlement inducer	3
I.B	SI solution	I	# spat; distance from spat to source of settlement inducer	3
II.B	SI solution	II	# spat; distance from spat to source of settlement inducer	3
III.B	SI solution	III	# spat; distance from spat to source of settlement inducer	3
IV.B	SI solution	IV	# spat; distance from spat to source of settlement inducer	3
V.B	SI solution	V	# spat; distance from spat to source of settlement inducer	3
VI.B	SI solution	VI	# spat; distance from spat to source of settlement inducer	3

Table 1. Overview of the experiments carried out in the microcosms.

microcosm we pasted one adult oyster on one of the sticks and placed it on the same position in each of the three microcosms. The fourth microcosm was used as a control situation (no oyster was added). In each microcosm we brought 20.000 oyster larvae. After 24 hours all sticks were taken out of the microcosms and oyster larvae settled on the sticks (spat) were counted under a Wild-Heerbrugg binocular with a magnification of 120 x (10 x 12). This experiment was repeated six times with only the position of the adult oyster in the microcosm changing. After every experiment we removed the marine cement of all the sticks and we covered every stick with a new layer of marine cement. By repeating the same experiment with the adult oyster on different at random positions we hoped to show that currents in the microcosm and other variables influencing the movement of the oyster larvae were not affecting the settlement behaviour. This was tested by a comparison of the measurements of each individual experiment (settlement in 1 microcosm) with the mean measurement of a number of related experiments or all experiments (n=6 x 3 replicas =18) making use of a χ^2 test and a Kruskal Wallis ANOVA by ranks. The correlation between the number of oyster larvae settled and their distance to the source of settlement inducer was calculated using a Spearman rank order correlation.

The six experiments described were repeated with a drip, releasing in drops the SI solution for a period of 24 hours, replacing the live adult oyster. This gives us the possibility to check whether the adult oyster is attracting the oyster larvae in an other way than through the chemical inducer (e.g. pumping activity of the oyster).

A second set of experiments was performed in the natural habitat of *S. cucullata*: the mangrove forest. With those experiments we hoped to find answers to two questions: (1) what is the influence of aggregation of adult *S. cucullata* oysters on the number of oyster larvae settling, and (2) has aggregation of adult *S. cucullata* oysters an effect on the survival rate of *S. cucullata* larvae. Table 2 summarises the experimental set-up of this second series of experiments, carried out in the mangrove ecosystem of Gazi Bay. Six experiments were carried out in Gazi Bay. In a first experiment we pasted an adult oyster on an empty trunk of a *Rhizophora mucronata* tree in the mid littoral zone. No other oysters were found in a radius of more than ten meters. Three days after pasting the oyster on the tree we released 100 oyster larvae in the immediate neighbourhood of the adult oyster when the tidal cycle reached its highest level. After another five days oyster spat was counted on the tree

N° of Experiment	Substrate	N° of adult oysters	Time after which oyster spat was counted (days)	N° of replicas	Removal of oysters from substrate	Spat survival on tree with median settlement
1	mangrove tree	1	5	10	(-)	(+)
2	mangrove tree	1	5	10	(+)	(+)
3	mangrove tree	(*)	5	10	(-)	(+)
4	mangrove tree	(*)	5	10	(+)	(+)
5	tile	1	5	10	(-)	(+)
6	tile	1	5	10	(+)	(+)

Table 2. Overview of the experimental set-up of the experiments carried out in natural conditions. (*) indicates that the tree was covered for more than 80% with oysters.

trunk where the oyster was pasted and on the tree trunks in the immediate neighbourhood. We repeated the experiment 10 times on 10 different places in the mangrove wood but every time on the same height above datum. On the tree with median settlement all settled oyster larvae (spat) were marked and numbered. We followed their survival over a period of 3 months. A second experiment was identical to the first one. However, the adult oyster was removed just after counting the spat. This makes it possible to study the influence of the proximity of an adult oyster on the survival rate of just settled oyster larvae.

Experiment 3 and 4 were identical to the first two. However, the mangrove tree with 1 adult oyster was replaced by a natural occurring mangrove tree covered for more than 80% by adult *S. cucullata* specimens. A comparison between experiment 3 and 4, and the first two experiments can tell us something more on the usefulness of aggregations of adult *S. cucullata*.

Experiment 5 and 6 were again identical to the first two with the only difference that the substrate on which the adult oyster was pasted was not a mangrove tree but a cemented tile, identic to the ones used in the oyster cultures along the Kenyan coast. A comparison with the first two experiments will give us an idea whether we can extrapolate our measurements under natural conditions to 'culture conditions'.

For each of the six experiments we carried out a control experiment. Control experiments were carried out at the same time as the original experiments but without the release of oyster larvae at day three of the experiments. This made it possible to evaluate the influence of natural settlement on our exper-

iments.
Oyster settlement was compared between each pair of the 6 experiments making use of a χ^2 test for 2 independent variables. Survival rate of the settled oysters was studied making use of Kaplan-Meier survival analyses. Survival curves were compared making use of Gehan's Wilcoxon test (Gehan, 1965).

Results

Figures 2a and b show the results of the experiments carried out in the microcosm. The number of oyster larvae settled (minimum, maximum and median counts) was related to the distance between the place of larval settlement and the place of release of the settlement inducer (live adult oyster or a fluid containing the chemical compounds responsible for settlement).
A χ^2 test comparing the number of oyster larvae settled on each distance smaller than 60 cm from the source of the settlement inducer with the mean number of oyster larvae settled on each distance gives χ^2 values ranging between 529.1 and 2461.0 ($df=8$, $p<0.001$). Those figures indicate significant differences between the different experiments. This was even the fact between replicate experiments.
A Kruskal-Wallis ANOVA by ranks showed no significant differences between any off the experiments, even after pooling all data with respect to origin of the chemical inducer or the place of release of the inducer. All experiments carried out with live adult oysters as the source of the settlement inducer showed no significant differences to each other (Kruskal-Wallis test: $H=0.031$, $df=5$, $N=288$; $p>0.999$). Similar results (Kruskal-Wallis test: $H=0.036$, $df=5$, $N=288$; $p>0.999$) were obtained when all experiments where the SI-solution was used were pooled. Comparison between both groups of treatment (live adult oyster and SI-solution) showed, again, no significant differences (Kruskal-Wallis test: $H=0.017$, $df=1$, $N=576$; $p>0.895$). We found a significant negative correlation between the number of oyster larvae settled and the distance from the source of the settlement inducer (Spearman rank order correlations: $R=-0.955$, $N=576$, $p<10^{-6}$).
Table 3 shows the median, minimum and maximum counts of the oyster larvae, settled 5 days after their release, in each experiment carried out in 'natural' conditions in Gazi Creek. This table also shows the results of the con-

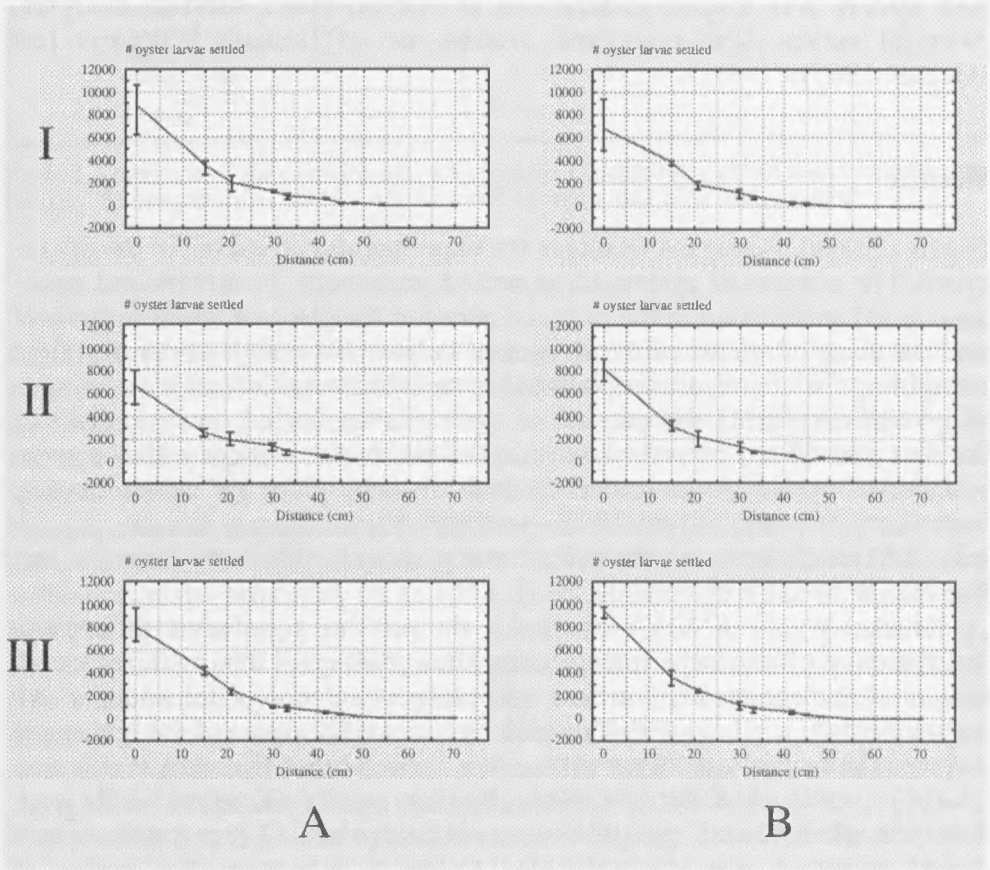

Figure 2a. Relation between the number of oyster larvae settled (median, maximum and minimum counts) and the distance between the place of larval settlement and the place of release of the settlement inducer. Column A and B show the results of different sources of settlement inducer (A: adult oyster; B: SI solution). The numbers I to III indicate different places in the microcosm where the settlement inducer was released (see fig. 1)

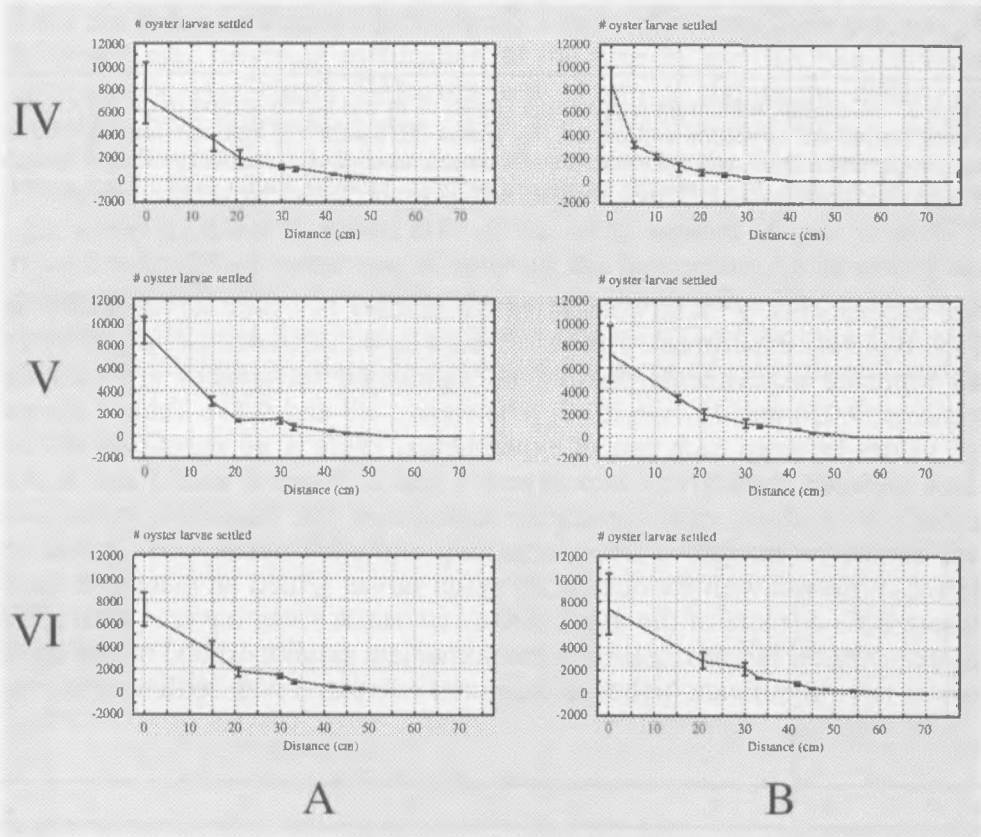

Figure 2b. Relation between the number of oyster larvae settled (median, maximum and minimum counts) and the distance between the place of larval settlement and the place of release of the settlement inducer. Column A and B show the results of different sources of settlement inducer (A: adult oyster; B: SI solution). The numbers IV to VI indicate different places in the microcosm where the settlement inducer was released (see fig. 1)

Exp. N°	Min.	Max.	Median	N_E	Max. Control	N_C
1	35	59	46	10	2	10
2	32	58	48	10	0	10
3	67	92	77	10	0	10
4	69	95	86	10	3	10
5	23	37	28	10	1	10
6	22	39	29	10	1	10

Table 3. Minimum, maximum and median counts of oyster larvae settled in the six experiments carried out in natural conditions. N_E shows the number of replicate samples within each experiment. Max. control shows the maximum number of oyster larvae settled in each control experiment. N_C gives the number of replicate samples within each control experiment.

trol experiments. χ^2 tests comparing the number of oyster larvae settled in each replicate experiment with the mean number of oyster larvae settled in all replicate experiments showed no significant differences (χ^2 ranging between 9.024 and 13.769; df=9, p between ≤.43 and ≤.13). Table 4 shows χ^2 values between each pair of experiments. There is no significant difference between respectively experiment 1 and 2, 3 and 4, and, 5 and 6. All other combinations show significant differences. The number of oyster larvae settled on mangrove trees containing one adult oyster was significant lower compared with the number of oyster larvae settled on mangrove trees where 80% or more of the trunk and/or root surface was covered with adult oysters. Oyster larvae released in the immediate neighbourhood of one adult oyster have significant higher settling rates compared with settlement on the

Exp. N°	1	2	3	4	5	6
1		0.0004	7.0368**	9.5080**	3.9310*	3.7354*
2			6.9110**	9.3867**	4.0106*	3.8131*
3				0.1941	20.6884**	20.2660**
4					24.5972**	24.1428**
5						0.0026
6						

*: $p \leq 0.05$
**: $p \leq 0.01$

Table 4: Comparison of the number of oyster larvae settled between all combinations of experiments carried out in natural conditions making use of a $\chi 2$ test for 2 independent samples. $\chi 2$-values indicating significant ($p \leq 0.05$) and highly significant ($p \leq 0.01$) differences are marked with respectively (*) and (**).

oyster rack tiles.

In each of the experiments oyster larvae settled on the mangrove tree with median settlement were used in a survivorship experiment. Kaplan and Meier Survivorship functions of oyster spat up to three months after settlement are shown in Figure 3 to 5. Figure 3 shows the cumulative percentage of oyster spat surviving in function of the time in the first two survival experiments. A Gehan's Wilcoxon test shows a significant difference between both survival curves (Gehan's Wilcoxon test statistic =4.96, $p<10^{-6}$). Experiment 1 shows no mortality during the first two months. However, within the last 25 days of the experiment the percentage of oyster spat surviving drops to almost 60% of the initial amount of spat. In experiment 2 only 55% of oyster spat is surviving the first month. At the end of the experiment only 25% of the initial oyster spat is surviving. This is less than half the oyster spat surviving experiment one after three months. Figure 4 shows the Kaplan and Meier Survivorship functions of experiment 3 and 4.

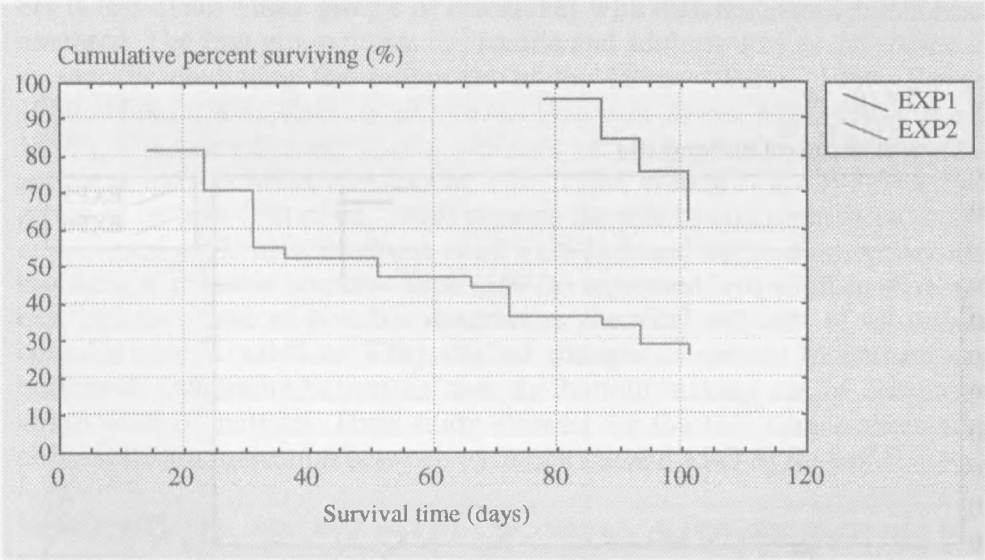

Figure 3. The cumulative percentage of oyster spat surviving in function of the time (survival experiment 1 and 2). Gehan's Wilcoxon test shows a significant difference ($p\leq10^{-6}$) between both survival curves.

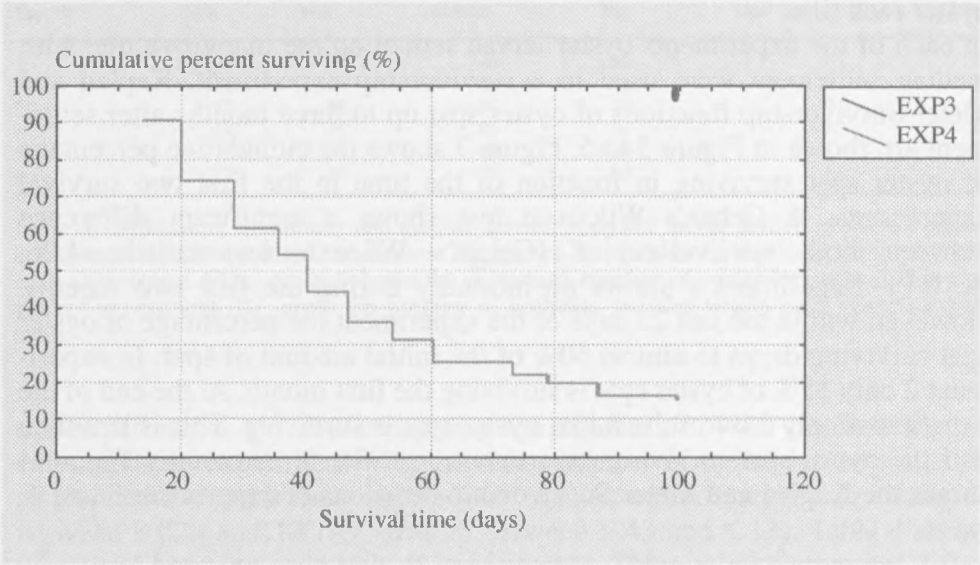

Figure 4. The cumulative percentage of oyster spat surviving in function of the time (survival experiment 3 and 4). Gehan's Wilcoxon test shows a significant difference ($p \leq 10^{-6}$) between both survival curves.

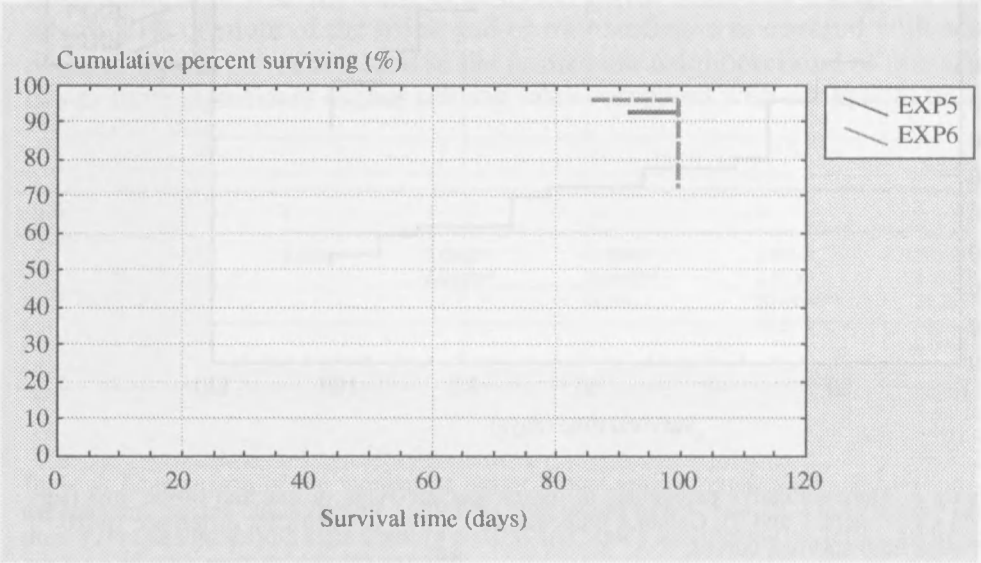

Figure 5. The cumulative percentage of oyster spat surviving in function of the time (survival experiment 5 and 6). Gehan's Wilcoxon test shows a significant difference ($p \leq 10^{-6}$) between both survival curves.

Both curves are significant different (Gehan's Wilcoxon test statistic =9.74, $p<10^{-6}$). In experiment 3 almost all the oyster spat is surviving, while in experiment 4 the oyster spat survival drops as low as 20% of the original amount of oyster larvae settled. 50% of the initial oyster spat of experiment 4 did not survive the first month. In Figure 5 the survival of oyster spat in function of the time in experiment 5 and 6 is shown. A Gehan's Wilcoxon test shows no significant difference between both survival curves (Gehan's Wilcoxon test statistic =1.92, $p \leq 0.552$). However, Figure 5 shows a substantial drop in the survival rate of experiment 6 on the last day of the experiment.

Discussion and conclusion

Cole and Knight-Jones (1939) were the first to describe the gregarious settlement of oyster larvae. Since that moment scientists are in search of the mechanisms involved in gregarious settlement. Most researchers agree on the involvement of chemical inducers. The source of those chemical inducers is less clear. Three groups of researcher with different view points have emerged. The first group points to juvenile and adult oysters as the source of substances mediating the settlement of the larvae (Walne, 1966; Bayne, 1969; Hidu, 1969; Keck *et al.*, 1971; Veitch & Hidu, 1971, Hidu *et al.*, 1978). The second group thinks biofilms on oyster shell surfaces are the source of inducer molecules (Bonar *et al.*, 1986; Weiner *et al.*, 1989). A third group of authors (Fitt *et al.*, 1989) stresses the role of ammonia as an inducer of larval settlement.Tamburri *et al.* (1992) found both adult oysters and biofilms as possible sources. Tack (1997a) separated live adult oysters and biofilms as source of possible waterborne chemical inducers of settlement behaviour in *S. cucullata*. They studied changes in vertical movement and horizontal swimming behaviour near the bottom making use of computer-video motion analyses. Their study showed for the first time a three way chemical communication between oyster larvae and adult oysters of *S. cucullata*.

In this study we used two sets of experiments. A first one made use of a microcosm. The second of the natural mangrove biotope. The microcosm was nothing more than a very rough copy of the mangrove ecosystem. The use of such a microcosm has one enormous advantage: the possibility to reg-

ulate the distance between two possible settling substrates of *S. cucullata*. The microcosm experiments show the relation between the number of oyster larvae settled and the distance between the source of the inducer mediating settlement and the place of the actual settlement (Fig. 2a and b). This relation was studied under different research conditions: different sources of the chemical inducer, different places of release of the chemical inducer, ... However, χ^2 tests showed even significant differences between the so called replicas taken. We speak about so called 'replicas' because in reality not all the experiments were carried out with all variables constant. In the first six experiments the sources of the chemical inducer mediating settlement were live adult oysters. All replicas made use of a different adult oyster. Wo have no idea what the variation is between the SI release of the different oysters used in this experiment. This variation can explain why the results of the replicate samples are significant different. However, in the second set of six experiments, where the IS solution was used, the same differences were found, while concentrations released by the drips were the same in all experiments. Another explanation for the differences seen are external variables influencing the chance an oyster larva comes close to the source of the settlement inducer. This depends especially on the currents (Palmer and Strathmann, 1981; Levin, 1984). We were not able to measure those currents on a micro scale, let alone to compare them between the different microcosms. The major implication of those differences between the 'replicas' is the impossibility to compare the different experiments in the first part of this study. Even if we would find significant differences we would not be able to find out whether those differences originate from the different experimental set up or from the differences seen between the replicas. In the results section we already mentioned that we only used distances closer than 60 cm to the origin of the chemical inducer. A distance of 60 cm (in the microcosm environment with one adult oyster present) seems to be the maximum distance from its source at which the settlement inducer is still active. Including distances > 60 cm would lead to the impossibility to use the χ^2 test. This kind of test does not allow more than 20% of the expected values to be less than 5, and no expected frequency should be less then 1 (Cochran, 1954).
The only variable of which we were able to show its influence on the settlement of *S. cucullata* larvae is the distance between the place of settlement and the original source of the settlement inducer. This view is supported by the results of Spearman rank order correlations showing significant correla-

tions between the number of oyster larvae settled and the distance from the source of the settlement inducer to the place where actual settlement takes place. In Figure 2a and b we can see the number of oyster larvae settled is very high in the immediate neighbourhood of the inducer source and diminishes very quickly when the distance between the place of settlement and the origin of the settlement inducer becomes larger. Tack (1997a) showed the necessity of a minimum concentration of the settlement inducer to trigger settlement. The experiments in the microcosm support this view. The closer you are to the release point of the chemical inducer, the higher the concentration of the settlement inducer, the higher the number of oyster larvae settled. The more or less exponential decrease of oyster larvae settling when moving away from the inducer source can explain why in natural conditions *S. cucullata* is only found on a few trees in very large numbers.

The main aim of our experiments in the mangrove biotope was to study the influence of aggregations of adult *S. cucullata* specimens on the settling behaviour and on the survival of *S. cucullata* larvae.

In the first part of the experiment we studied the number of oyster larvae settling. In the second part we had a look at the influence of aggregations of adult *S. cucullata* specimens on the survival rate of the oyster spat.

No significant differences are seen between respectively experiment 1 and 2, 3 and 4, and 5 and 6. Up to the moment of settling those groups of experiments were replicates of each other. More interesting are the significant differences between the other experiments. Aggregation of adult *S. cucullata* specimens leads to a significant higher settlement of oyster larvae compared with settlement in the neighbourhood of a single adult conspecific. Higher concentrations of the settlement inducer are probably the major reason for this difference.

We found a significant difference between oyster settlement in the neighbourhood of a stand alone oyster on a natural substratum compared with the substratum used in the oyster culture. Baker (1992) found oyster larvae settling more onto rough than smooth surfaces. This can explain the lower settlement in experiments 5 and 6 compared with the other experiments.

Kaplan and Meier Survivorship functions were used to study the role of the adult oysters after settlement of the oyster larvae. Comparisons between the different experiments were made making use of the Gehan's Wilcoxon test. This test was used because of the relative small samples used (occasionally under N=50) and because equal hazard risk throughout the period of the

experiments could not be expected (Gehan, 1965). In the first two experiments survival of oyster larvae is significant higher in the immediate neighbourhood of an adult conspecific compared with larval survival in absence of such a specimen. Field observations on numerous occasions showed crab predation as the main cause of death in experiment 2. Crabs were especially active at high tide. The difference between the survival curves of experiment 3 and 4 is even more obvious. Almost 100% of the oyster larvae are surviving in the neighbourhood of an aggregation of adult conspecifics while only 20% of the oyster spat is surviving in a situation where no adult specimens are available. Again, field observations show crab predation as main cause of mortality of oyster spat. Because of the important role crabs were playing in the mortality of oyster spat we counted their numbers on the mangrove trees involved in the experiments. A χ^2 test showed no significant differences in the number of crabs between experiments with high and low spat mortality. A possible hypothesis explaining those differences is the production of a chemical substance by *S. cucullata* to keep certain species of crabs on a safe distance from the oyster spat. Increased protection from predation and reduced juvenile and adult mortality were already described by Sebens (1983), Keough (1984), Knight-Jones (1951), Highsmith (1982) and Young (1983) as advantages of gregarious settlement leading to aggregation.
Between experiment 5 and 6 the Gehan's Wilcoxon test does not find a significant difference. On the oyster racks used in this study no crabs were found.
This study shows large differences in numbers of oyster larvae settling on certain distances from the source of the settlement inducer, even between 'replicate' samples. However, we found a significant correlation between the distance from the source of the inducer to the place of settlement and the number of oyster larvae settled. High settlement is seen in the immediate neighbourhood of the source of the settlement inducer. The number of oyster larvae settling decreases more or less exponentially with the distance from the source. In natural conditions a maximum settlement is seen on substrates with large aggregates of adult conspecifics. Substrates with only one adult oyster (man made situation) were less efficient in attracting oyster larvae. Oyster spat has a higher rate of survival in the neighbourhood of aggregates of adult *S. cucullata* specimens compared with the rate of survival in the neighbourhood of a single adult oyster. Survival of oyster spat without adult specimens in the neighbourhood is very low in natural conditions. Crab

predation seems to be the major cause of death. The production of a chemical compound to keep spat eating crabs on a distance can be a possible hypothesis to explain differences between the survival rates of the different experiments.

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References

Baker, P. (1992). Roles of physical cues in oyster larval settlement site choice. J. Shellfish Res. 11: 189

Bayne, G. L. (1969). The gregarious behavior of the larvae of *Ostrea edulis* L. at settlement. J. Mar. Biol. Assoc. U.K. 49: 327-356

Bonar, D. B., Weiner, R. M., Colwell, R. R. (1986). Microbial-invertebrate interactions and potential for biotechnology. Microb. Ecol. 12: 101-110

Burke, R. D. (1986). Pheromones and gregarious settlement of marine invertebrate larvae. Bull. Mar. Sci. 39: 323-331

Buss, L. W. (1981). Group living, competition, and the evolution of cooperation in a sessile invertebrate. Science 213: 1012-1014

Cochran, W. G. (1954). Some methods for strengthening the commen χ^2 tests. Biometrics 10: 417-451

Cole, J. A., Knight-Jones, E. W. (1939). Some observations and experiments in the setting behavior of *Ostrea edulis*. J. Cons. Int. Explor. Mer. 14: 86-105

Crisp, D. J. (1974). Factors influencing settlement of marine invertebrate larvae. In: Grant, P. T., Mackie A. M. (Ed.) Chemoreception in Marine Organisms. Academic Press, London, p. 177-265

Denny, M. W., Shibata, M. F. (1989). Consequence of surf-zone turbulence for settlement and external fertilization. Am. Nat. 134: 859-889

Fitt, W. K., Coon, S. L., Walch, M., Weiner, R. M., Colwell, R. R., Bonar, D. B. (1990). Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. Mar. Biol. 106: 389-394

Fitt, W. K., Labare, M. P., Fuqua, W. C., Walch, M., Coon, S. T., Bonar, D. B., Colwell, R. R., Weiner, R. M. (1989). Factors influencing bacterial production of inducers of settlement behavior of larvae of the oyster *Crassostrea gigas*. Microb. Ecol. 17: 287-298

Gehan, E. A. (1965). A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52: 15-21

Gotelli, N. J. (1990). Stochastic models of gregarious larval settlement. Ophelia 32: 95-108

Grosberg, R. K. (1987). Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. Evolution 41: 372-384

Hidu, H. (1969). Gregarious setting in the American oyster, *Crassostrea virginica* Gmelin. Chesapeake Sci. 10: 85-92

Hidu, H., Valleau, W. G., Veitch, F. P. (1978). Gregarious setting in the European and American oysters-response to surface chemistry vs. waterborne pheromones. Proc. Nat. Shellfish. Assoc. 68: 11-16

Highsmith, R. C. (1982). Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. Ecology 63: 329-337

Hughes, R. N. (1978). Coloniality in *Vermetidea* (Gastropoda). In: Larwood, G., Rosen, B. R. (Ed.) Biology and Systematics of Colonial Organisms. Academic Press, New York, p. 319-327

Keck, R., Maurer, D., Kauer, J. C., Sheppard, W. A. (1971). Chemical stimulants affecting larval settlement in the American oyster. Proc. Nat. Shellfish. Assoc. 61: 24-28

Keough, M. J. (1984). Kin-recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* (L.). Evolution 38: 142-147

Knight-Jones, E. W. (1951). Gregariousness and some other aspects of the settling behavior of *Spirorbis*. J. Mar. Biol. Assoc. U.K. 30: 201-222

Levin, L. A. (1984). Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbances. Ecology 65: 1185-1200

Levitan, D. R. (1991). Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biol. Bull. 181: 261-268

Loosanoff, V. L., Davis, J. C. (1963). Rearing of bivalve mollusks. In: Russell, F. S. (Ed.) Advances in Marine Biology. Academic Press, London, p. 1-136

Meadows, P. S., Campbell, J. I. (1972). Habitat selection by aquatic invertebrates. Adv. Mar. Biol. 10: 271-282

Palmer, A. R., Strathmann, R. R. (1981). Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. Oecologia 48: 308-318

Pawlik, J. T., Hadfield, M. G. (1990). A symposium on chemical factors that influence the settlement and metamorphosis of marine invertebrate larvae: introduction and prospective. Bull. Mar. Sci. 46: 450-454

Pennington, J. T. (1985). The ecology of fertilization of echinoid eggs: the consequence of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. 169: 417-430

Sebens, K. P. (1983). Larval and juvenile ecology of the temperate octocoral *Alcyonium siderium* Verrill II. Fecundity, survivorship, and juvenile growth. J. Exp. Mar. Biol. Ecol. 72: 263-285

Tack, J. F. (1997a). Chemical inducers mediating settlement of *Saccostrea cucullata* (von Born, 1778) larvae. In: Behavioural aspects of the mangrove oyster *Saccostrea*

cucullata (von Born, 1778) explaining its macro and micro distribution along the Kenyan coast. Unpublished Ph.D. thesis, Vrije Universiteit Brussel, Brussels, p. 167-192

Tack, J. F. (1997b). Spawning behaviour and larval culture of *Saccostrea cucullata* (von Born, 1778). In: Behavioural aspects of the mangrove oyster *Saccostrea cucullata* (von Born, 1778) explaining its macro and micro distribution along the Kenyan coast. Unpublished Ph.D. thesis, Vrije Universiteit Brussel, Brussels, p. 157-166

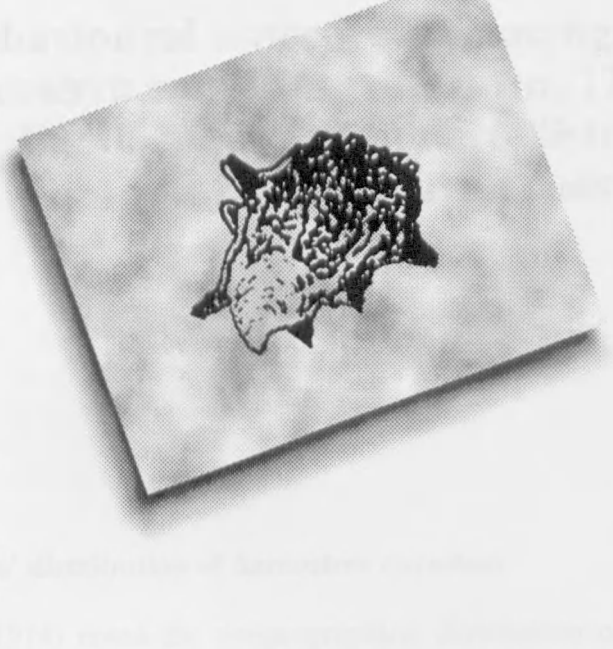
Tamburri, M. N., Zimmer-Faust, R. K., Tamplin, M. L. (1992). Natural Sources and Properties of Chemical Inducers Mediating Settlement of Oyster Larvae: A Re-examination. Biol. Bull. 183: 327-338

Veitch, F. P., Hidu, H. (1971). Gregarious setting in the American oyster *Crassostrea virginica* Gmelin: I. Properties of a partially purified "setting factor". Chesapeake Sci. 12: 173-178

Walne, P. R. (1966). Experiments in large-scale culture of the oyster *Ostrea edulis*. Fish. Invest. Lond. Ser. 2 25: 1-53

Weiner, R. M., Walch, M., Labare, M. P., Bonar, D. B., Colwell, R. R. (1989). Effect of biofilms of the marine bacterium *Alteromonas colwelliana* (LST) on the set of the oyster *Crassostrea gigas* (Thunberg, 1793) and *C. virginica* (Gmelin, 1791). J. Shellfish Res. 8: 117-123

Young, C. M. (1983). Functional significance of larval behavior in an intertidal population of the ascidian *Pyura haustor*. Am. Nat. 23: 1021



Chapter

6

EPILOGUE

Behavioural aspects of the mangrove oyster *Saccostrea cucullata* (von Born, 1778) explaining its macro and micro distribution along the Kenyan coast.

Epilogue

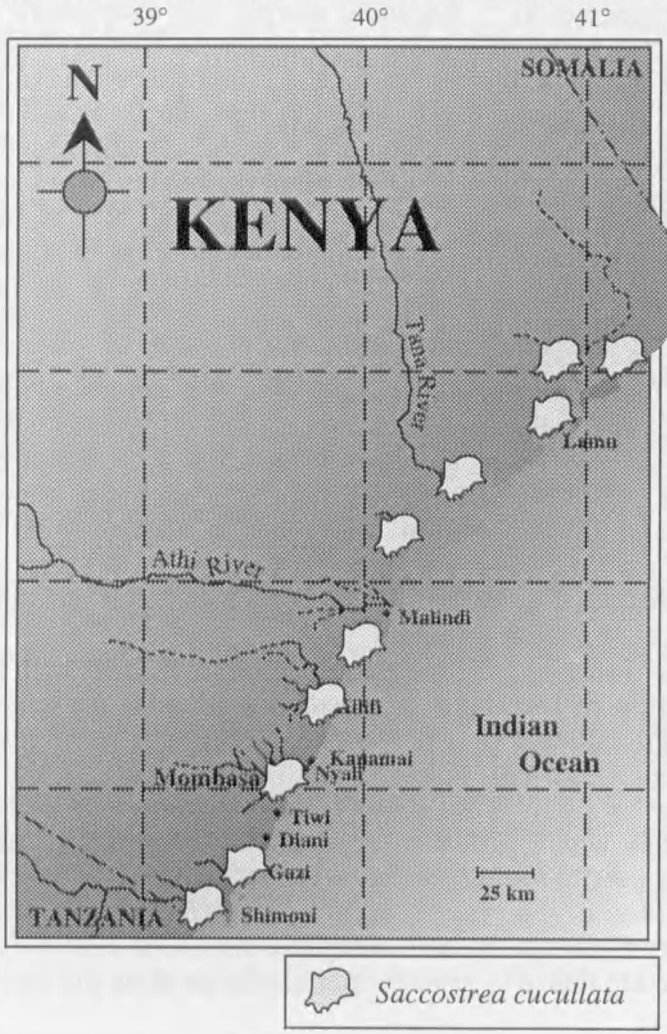
'Macro' distribution of *Saccostrea cucullata*

Day (1974) noted the zoogeographical distribution of *S. cucullata* in the western part of the Indian Ocean. Ecological studies of this species have been carried out over the entire region: Seychelles (Taylor, 1968), Aldabra (Taylor, 1970), Tanzania (Hartnoll, 1976), Somalia (Chelazzi and Vannini, 1980) and Kenya (Ruwa, 1984, 1990; Okemwa *et al.,* 1986; Tack, 1992; Tack *et al.*, 1992). Figure 1 shows the 'macro' distribution of *S. cucullata* along the Kenyan coast. A majority (87%) of all *S. cucullata* is found on the trunks and stilt roots of mangrove trees. *S. cucullata* is also found on rocky substrata (9%), but always within a range of 5 km from the nearest mangrove forest. The remaining 4% are specimens growing on other substrates available in the tidal zone. Oysters do not grow on mud bottoms except where such firm and not mud covered substrata as gastropod shells are available. One of the largest beds of *S. cucullata* is that found on the reef extending from Ras Ngomeni, north of Malindi (Kenya). But even in this case the man-

grove forest is near.

Saccostrea cucullata (von Born, 1778) is an edible oyster found in the upper littoral zone, following the terminology of Lewis (1964) and Hartnoll (1976). The oysters incrust the mangrove stems and stilt roots only in a narrow band up to 4 m wide at the edges of the swamps facing open water of tidal channels or of the centre of the lagoon (Van Someren and Whitehead, 1961). Below low tide level the oysters remain covered by water continuously and therefore, remain exposed to such predators as crabs and fish. These predators are so numerous that they eliminate all young and thin-shelled oysters (Stenzel, 1971). Above average high tide level oysters become exposed to air and sunshine too long to survive; only barnacles can survive there.

Walsh (1974) suggested the existence of extensive mangal depended upon five basic factors. Chapman (1975, 1977, 1984) believed there are seven: (1) air temperature, (2) ocean currents, (3) protection from wave action, (4) shallow shores, (5) salt water, (6) tidal range, and (7) substrate. Mangrove distribution in the tropics and subtropics is often linked with the presence of estuaries and creeks (Macnae, 1968; Barth, 1982; Blasco, 1991). There is a consensus that the brackish water micro environment, which is the key factor for the development of mangroves, is caused by river discharges into the oceans (Macnae, 1968; Barth, 1982; Snedaker, 1982). However all over the world mangrove areas are found where no rivers or estuaries are in the immediate neighbourhood. Chapter 3 explains how this is possible. The coastal zone of Kenya is characterised by moderately high groundwater flow ranging between 0.31 and 12.8 $m^2.day^{-1}$ (average value: 4 $m^2.day^{-1}$). The high groundwater flow is due to high potential gradients, high infiltration capacity of the geologic formations and high precipitation received by the area. High discharge of fresh groundwater into the sea is in conformity with Cashwell and Baker's (1953) assertion that close to the shores, in some points, seepage of freshwater occurs. Seawater seepage is reported in several areas along the Kenyan coast (Isaac and Isaac, 1968; Knutzen and Jasuund (1979); Ruwa and Polk, 1986). Ruwa (1993) describes considerable discharges of fresh water from the underground aquifers in those regions where this Ph.D. study finds high groundwater flow. In chapter 3 we showed a clear relation between the distribution of the mangrove ecosystem in Kenya and regions with a groundwater flow of at least $1m^2.day^{-1}$. To establish the effects of groundwater flow along the Kenyan coast on the relative frequen-



Figuur 1. 'Macro' distribution of *Saccostrea cucullata* along the Kenyan coast.

cy, the mean maximum tree height, the above-ground biomass.m^{-2} and the number of propagules.m^{-2}, *Avicennia marina* and *Rhizophora mucronata* were studied in different mangrove areas along the Kenyan coast. Because of its double zonation *A. marina* was studied at the landwards and at the seawards end of the mangrove forest. High groundwater flow favours competitors of *A. marina* in the colonisation of the shore. Higher groundwater flow leads to mangrove trees with a higher mean maximum length and a higher above-ground biomass. The number of propagules.m^{-2} decreases with increasing groundwater flow. This is probably an effect of higher survival rates of the propagules in areas with a lower salinity. *A. marina* shows a number of adaptations to the dryer, more saline conditions on the land side of the mangrove forest.

Groundwater discharge along the coastal zone also influences the spawning behaviour of *S. cucullata*. In chapter 5 significant differences were observed in spawning efficiency among adult oysters kept in seawater of different salinities. The lower the salinity, the higher the spawning efficiency. Salt stress is probably the explanation. The spawning efficiency did not change in function of the temperature. In natural conditions *S. cucullata* spawns during the rainy season. This certainly leads to an increase of the number of larvae produced by the adult oysters.

We related the distribution of the mangrove forest to areas with a minimum groundwater flow of $1 m^2.day^{-1}$. *S. cucullata* is found in the same areas. The relative high groundwater flow in those regions influences the spawning efficiency of *S. cucullata*. This makes it possible for *S. cucullata* to maintain a certain population size in those regions. We did not test in this study whether higher salinities would influence survival of oyster larvae. Anyhow, the chances on successful settlement depends on the number of larvae available. This number increases in regions with maximal groundwater flow, resulting in groundwater discharge. So both mangrove distribution and the distribution of *S. cucullata* are linked to groundwater discharge along the Kenyan coastal zone.

Maybe, the close relation between the distribution of the mangrove oyster *S. cucullata* and its preferential habitat, the mangrove forest, finds its origin in the feeding behaviour of this species. In chapter 4 we have seen that *S. cucullata* takes up large amounts of DOC in the size range 0.2 to 1.2 µm in the first 2 hours the oyster is submerged. The most important part of this DOC consists of colloidal particles and bacteria. S. *cucullata* shows a clear pref-

erence to feed on bacteria. Also the saltmarsh mussel *Geukensia demissa* was capable of the efficient clearance of natural bacterioplankton (Wright *et al.*, 1982). Mwangi (1994) observed a reduction of bacterial biovolume in Gazi Creek due to *S. cucullata* feeding. However, due to the large concentrations of colloidal particles in the water, this food source is the most important one for the mangrove oyster. The colloidal material shows a comparable $\delta^{13}C$ value with the $\delta^{13}C$ values of seagrasses (Woitchik *et al.*, 1993). Between the third and the fourth hour *S. cucullata* shifts from a preferential clearance of the DOC component towards a preferential clearance of the POC component of the food. Besides phyto and zooplankton *S. cucullata* takes up large seston particles originating from mangrove detritus. The uptake of POC particles is related to its diameter. The longer the submersion time the greater the diameter of the particles taken up by *S. cucullata*. Summarised we can state that the main food source of *S. cucullata* is DOC. With increasing submersion times there is a shift from seagrasses towards mangrove detritus as main DOC source for *S. cucullata*. This indicates that the mangrove forest as is not the only food source of *S. cucullata*.

Concerning the 'macro' distribution of *S. cucullata* we can come to the following conclusions:

- the mangrove forests are the main sources of substrate in the intertidal zone;
- groundwater flow influences mangrove distribution and the number of oyster larvae spawned by adult oysters. This makes it possible for *S. cucullata* to maintain its populations in areas with high groundwater outflow. Those areas are characterised by the presence of mangrove forest; and
- mangrove detritus is a food source for *S. cucullata*, becoming more important when the submersion time increases.

'Micro' distribution of *S. cucullata*

In chapter 2 variations of shell form and shell length were studied for oysters in the mangroves of Gazi Creek, and related to different environmental factors. We found a relation between shell form and substrate diameter, height above chart datum and orientation with respect to the tidal current. Shell length of *S. cucullata* was not influenced by density up to a cover of 70%. For densities higher than 70% there was a fairly strong negative correlation. More than 90% (unpublished data) of *S. cucullata* is found in clusters with a cover of more than 70%.

Aggregation of *S. cucullata* has a negative effect on shell length, and hence on shell growth. However, for sessile or sedentary species reproducing sexually by spawning gametes into surrounding waters aggregation resulting from larval settlement can be critical. Once the gametes are released in the surrounding waters the hydrodynamic properties of turbulent flowing water tend to dilute and disperse them. This decreases the likelihood of fusion between eggs and sperm. The same problem occurs when the distance between spawning individuals of opposite sexes is increasing (Pennington, 1985; Grosberg, 1987; Denny and Shibata, 1989; Levitan, 1991). Once external fertilisation occurs the embryos develop into larvae. They spend hours to months in the water column before metamorphosing into the juvenile form. Quite often the time spent in the water column is related to the lunar cycle. During this time larvae are often suspended and carried by ocean currents, serving as dispersal agent for the species (Palmer and Strathmann, 1981; Levin, 1984). Chapter 5 describes the tendency of *S. cucullata* larvae to sink once they reach the straight-hinge stage. This is coherent with the low number of oyster larvae > 70 µm in the water (Bollen, 1993). According to Galtsoff (1964) bivalve larvae in estuaries were found especially near to the bottom of the water column. Galtsoff (1964) suggested these changes in vertical position maintained the larvae in the estuary. Even the last case is not able to explain the gregarious settlement of *S. cucullata* larvae.

Gregarious settlement leading to aggregation among juvenile and adult conspecifics has a number of other advantages: increased protection from predation (Sebens, 1983; Keough, 1984), increased competitive ability (Buss, 1981), increased filter feeding efficiency (Hughes, 1978), and reduced juvenile and adult mortality (Knight-Jones, 1951; Highsmith, 1982; Young, 1983). Gregariousness is critical for many sessile and sedentary species. We

can expect specific actions that promote the active selection of settlement sites by larvae. In chapter 5 live adult oysters and biofilms were separated as possible source of waterborne chemical inducers of settlement behaviour in *Saccostrea cucullata*. Changes in vertical movement and horizontal swimming behaviour near the bottom were studied making use of computer-video motion analyses. For the first time a three way chemical communication between oyster larvae and adult conspecifics of *S. cucullata* is shown.
Adult oysters produce one or more chemical substances inducing oyster larvae to produce another (set of) chemical compound(s). This (those) chemical compound(s) induce(s) production of (a) certain metabolite(s) in adult oysters. This (those) metabolite(s) is (are) responsible for the inducement of settling behaviour in *S. cucullata* larvae. Our study gives a strong indication that (the) chemical compound(s) released by the adult oysters are two times the same but released in different amounts.
Chapter 5 also shows how such a settlement inducer released by adult oysters provokes gregarious settlement. Gregarious settlement of *S. cucullata* larvae leads to aggregation of adult conspecifics. Experiments show how an aggregation of adult *S. cucullata* specimens attracts significantly more oyster larvae compared with a single adult oyster or no oyster at all. Also the survival rate of oyster larvae settled in the immediate neighbourhood of such an aggregation is much higher than the survival rate of larvae settled close to a single adult oyster.

Concerning the 'micro' distribution of *S. cucullata* we can come to the following conclusions:

- the spawning efficiency increases with a decrease in salinity;
- gregarious settlement is the result of a three-way chemical communication between adult *S. cucullata* specimens and their larvae, leading to the production of a settlement inducer;
- gregarious settlement of *S. cucullata* larvae leads to aggregations of adult conspecifics; and
- aggregations of adult *S. cucullata* specimens attract large amounts of oyster larvae and increases the survival rate of larvae settled in the immediate neighbourhood.

References

Barth, H. (1982). The biogeography of mangroves. In: Sen, D. N., Rasjpurohit, K. S. (Ed.) Tasks for Vegetation Science 2. Dr. W. Junk Publishers, The Hague, p. 35-59

Blasco, F. (1991). Les mangroves. La Recherche 231: 444-453

Bollen, A. (1993). *Saccostrea cucullata* (von Born, 1778): Voedselaanwezigheid in een mangrovekreek (Gazi, Kenya). Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels

Buss, L. W. (1981). Group living, competition, and the evolution of cooperation in a sessile invertebrate. Science 213: 1012-1014

Cashwell, P. V., Baker, B. H. (1953). Geology of the Mombasa-Kwale Area. Bulletin Geol. Surv. Kenya, Report No. 24, Nairobi

Chapman, V. J. (1975). Mangrove biogeography. In: Walsh, G. D., Snedaker, S. C., Teas, H. J. (Ed.) Proceedings International Symposium on the Biology and Management of Mangroves, Honolulu, 1974. Vol. 1. Univ. of Florida, Gainesville, p. 3-22

Chapman, V. J. (1977). Ecosystems of the World. Vol. 1. Elsevier, Amsterdam

Chapman, V. J. (1984). Mangrove biogeography. In: Por, F. D., Dor, I. (Ed.) Hydrobiology of the Mangal. Dr. W. Junk Publishers, The Hague, p. 15-24

Chelazzi, G., Vannini, M. (1980). Zonation of intertidal molluscs on rocky shores of southern Somali. Estuar. coast. mar. Sci. 10: 569-583

Day, J. H. (1974). A guide to marine life of South African shores. Balkema, Cape Town

Denny, M. W., Shibata, M. F. (1989). Consequence of surf-zone turbulence for settlement and external fertilization. Am. Nat. 134: 859-889

Galtsoff, P. S. (1964). The American oyster *Crassostrea virginica* Gmelin. Fish. Bull. US Fish Wildl. Serv. 64: 369-371

Grosberg, R. K. (1987). Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. Evolution 41: 372-384

Hartnoll, R. G. (1976). The ecology of some rocky shores in tropical East Africa. Estuar. coast. mar. Sci. 4: 1-21

Highsmith, R. C. (1982). Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. Ecology 63: 329-337

Hughes, R. N. (1978). Coloniality in Vermetidea (Gastropoda). In: Larwood, G., Rosen, B. R. (Ed.) Biology and Systematics of Colonial Organisms. Academic Press, New York, p. 319-327

Isaac, W. E., Isaac, F. M. (1968). Marine botany of Kenya coast. 3: General account of environment, flora and vegetation. J. East Afr. Nat. Hist. Soc. Nat. Mus. 27: 7-28

Keough, M. J. (1984). Kin-recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* (L.). Evolution 38: 142-147

Knight-Jones, E. W. (1951). Gregariousness and some other aspects of the settling behavior of *Spirorbis*. J. Mar. Biol. Assoc. U.K. 30: 201-222

Knutzen, J., Jasuund, E. (1979). Note on littoral algae from Mombasa, Kenya. J. East Afr. Nat. Hist. Soc. Nat. Mus. 168: 1-4

Levin, L. A. (1984). Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbances. Ecology 65: 1185-1200

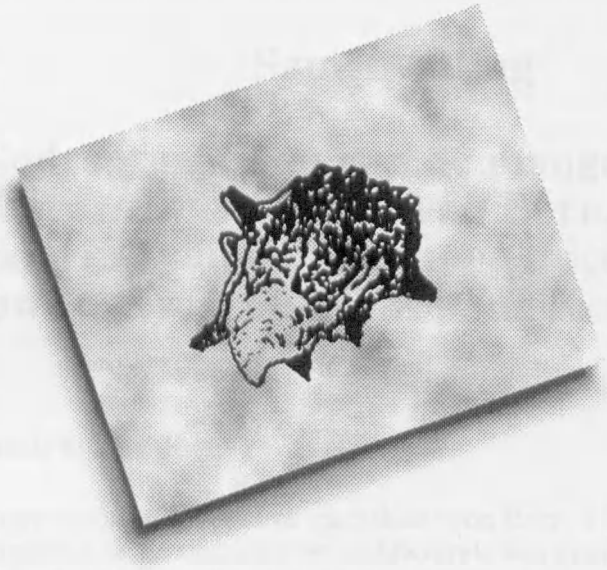
Levitan, D. R. (1991). Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biol. Bull. 181: 261-

268
Lewis, J. R. (1964). The ecology of rocky shores. Hodder & Stoughton, London
Macnae, W. (1968). A general account of the fauna and flora of mangrove swamps and forets in the Indo-West Pacific region. Adv. Mar. Biol. 6: 73-270
Mwangi, S. N. (1994). The influence of bivalve filter feeders on bacteria populations and seston in Gazi Bay and Wadden Sea. Unpublished. M. Sc. Thesis, Vrije Universiteit Brussel, Brussels
Okemwa, E. R., Ruwa, R. K., Polk, P. (1986). The autecology of the edible oyster *Crassostrea cucullata* Born, 1778: size-related vertical distribution at Mkomani, Mombasa. J. Sci. Ser. B 7: 9-14
Palmer, A. R., Strathmann, R. R. (1981). Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. Oecologia 48: 308-318
Pennington, J. T. (1985). The ecology of fertilization of echinoid eggs: the consequence of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. 169: 417-430
Ruwa, R. K. (1984). Invertebrate faunal zonation on rocky shores around Mombasa, Kenya. Kenya J. Sci. Ser. B 5: 49-65
Ruwa, R. K. (1990). Growth of *Crassostrea cucullata* Born (Bivalvia) at different levels in the intertidal zone. Aquaculture 88: 303-312
Ruwa, R. K. (1993). Zonation and distribution of creek and fringe mangroves in the semi-arid Kenyan coast. In: Lieth, H., Al Masoom, A. (Ed.) Towards the rational use of high salinity tolerant plants, Vol. 1. Kluwer Academic Publishers, The Hague, p. 97-105
Ruwa, R. K., Polk, P. (1986). Additional information on mangrove distribution in Kenya: some observations and remarks. Kenya J. Sci. Ser. B 7: 41-45
Sebens, K. P. (1983). Larval and juvenile ecology of the temperate octocoral *Alcyonium siderium* Verrill II. Fecundity, survivorship, and juvenile growth. J. Exp. Mar. Biol. Ecol. 72: 263-285
Snedaker, S. C. (1982). Mangrove species zonation: Why? In: Sen, D. N., Rasjpurohit, K. S. (Ed.) Tasks for Vegetation Science 2. Dr. W. Junk Publishers, The Hague, p. 111-125
Stenzel, H. B. (1971). Oysters. In: Moore, R. C. (Ed.) Treatise on Invertebrate Paleontology. Part N. Vol. 3. Mollusca 6. Bivalvia. Geological Society of America, Boulder, p. N953-N1224
Tack, J. F. (1992). Ecomorphology of the mangrove oyster *Saccostrea cucullata* (von Born, 1778). Academiae Analecta 54 (4): 1-20
Tack, J. F., Vanden Berghe, E., Polk, P. (1992). Ecomorphology of *Crassostrea cucullata* (Born, 1778) (Ostreidae) in a mangrove creek (Gazi, Kenya). Hydrobiologia 247: 109-117
Taylor, J. D. (1968). Coral reef associated invertebrate communities (mainly molluscan) around Mahe, Seychelles. Phil. Trans. r. Soc., Lond. Ser. B 254: 129-206
Taylor, J. D. (1970). Intertidal zonation at Aldabra atoll. Phil. Trans. r. Soc., Lond. Ser. B 260: 173-213
Van Someren, V. D., Whitehead, P. J. (1961). An Investigation of the Biology and Culture of an East African Oyster *Crassostrae cucullata*. Col. Off. Fish. Publ. 14: 2-41
Walsh, G. E. (1974). Mangroves: a review. In: Reinhold, R., Queen, W. (Ed.) Ecology of Halophytes. Academic press, New York, p. 51-74
Woitchik, A. F., Kazungu, J., Dehairs, F. (1993). Transfer of C from primary to secondary

and tertiary producers: preliminary results. In: Woitchik, A. F. (Ed.) Final Report Dynamics and Assessment of Kenyan Mangrove Ecosystems. ANCH, Vrije Universiteit Brussel, Brussels, p. 216-220

Wright, R. T., Coffin, R. B., Ersing, C. P., Pearson, D. (1982). Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27: 91-98

Young, C. M. (1983). Functional significance of larval behavior in an intertidal population of the ascidian *Pyura haustor*. Am. Nat. 23: 1021



SAMENVATTING

Samenvatting

Gedragsaspecten van de mangroveoester *Saccostrea cucullata* (von Born, 1778) ter verklaring van de macro- en microdistributie van deze soort langsheen de Keniase kust.

Hoofdstuk 1: Inleiding

De mangroveoester *Saccostrea cucullata* (von Born, 1778) komt voor in het getijdengebied op de stammen en luchtwortels van mangrovebomen. Oesters die zich onder het niveau van het laag water bevinden, en bijgevolg continu ondergedompeld zijn, blijven de gehele tijd blootgesteld aan predators, waaronder vis- en krabsoorten. De talrijke predators elimineren de jonge en fragiele oesters in een minimum van tijd. Boven de gemiddelde hoogwaterlijn blijven de oesters te lang blootgesteld aan de lucht en aan de zonneschijn. De mangroveoester groeit slechts op harde, slibvrije substraten.
Verschillende oestergenera hebben het mangrovebiotoop tot hun habitat gemaakt: *Crassostrea* door middel van *C. rhizophorae* (Guilding, 1828) in het Caraïbisch en het West-Indisch gebied; *Lopha* door middel van *L. folium* ecomorf *cristagalli* (Linné, 1758) in de regio tussen de Indische Oceaan en het zuidwesten van Japan; en *Saccostrea* door middel van *S. cucullata* (von Born, 1778) in tropisch West- en Oost-Afrika tot Honshu in Japan.
Er bestaat een duidelijke relatie tussen de verspreiding van het mangroveecosysteem en de verspreiding van *S. cucullata*. Het grootste deel (87%) van de mangroveoesters vinden we terug op de stammen en luchtwortels van de mangrovebomen. Ook op rotssubstraat vinden we deze soort terug (9%), maar steeds ligt het dichtstbijzijnde mangrovegebied op een afstand van

minder dan 5 km. De overige 4% groeit op harde substraten, zoals wrakken en schelpen, in de getijdenzone. Maar ook in dit laatste geval is het mangrovewoud nooit ver weg.
De vraag die zich stelt is of het mangrovebos, naast zijn taak van substraat nog een andere rol speelt in de levenscyclus van *S. cucullata*. In deze studie wordt nagegaan of het mangrovewoud een noodzakelijke voedingsbron vormt voor *S. cucullata*. Tevens wordt nagegaan of de verspreiding van *S. cucullata* enerzijds, en de distributie van het mangrovewoud anderzijds, geen gemeenschappelijke basis hebben.
De 'micro' distributie van *S. cucullata* is zeer opvallend. Bij een aantal mangrovebomen zijn de stam en de luchtwortels helemaal overgroeid met oesters, terwijl op het merendeel van de bomen helemaal geen oesters voorkomen. De larven van *S. cucullata* verblijven ongeveer 28 dagen in de waterkolom. Gedurende deze periode worden de oesterlarven verspreid over een groot gebied. Toch zullen ze zich uiteindelijk vasthechten in de buurt van een groep volwassen oesters. Deze studie gaat op zoek naar het mechanisme dat de oesterlarven de weg wijst naar hun volwassen soortgenoten.

Hoofdstuk 2: Ecomorfologie

In dit hoofdstuk wordt de invloed van omgevingsvariabelen op de grootte en op de vorm van *Saccostrea cucullata* (von Born, 1778) onderzocht.
In het eerste deel werd getracht om de oesterlengte te correleren met het soort mangroveboom, de diameter van de tak die dient als substraat, de benaderde procentuele oesterbegroeiing, hoogte boven de bodem, hoogte boven het referentieniveau en oriëntatie ten opzichte van de vloedstroming. Hiertoe werd gebruik gemaakt van de Kendall coëfficiënt voor rang correlatie. De correlatie van de oesterlengte met de hoogte boven het referentieniveau was negatief maar zeer laag. Wanneer echter alle metingen van oesters die lager dan 0.25 m boven de bodem voorkomen of met een procentuele oesterbegroeiing hoger dan 65% werden verwijderd uit de dataset dan steeg de correlatie drastisch (van $\tau = -0.092$ tot $\tau = -0.522$). De hoogte boven het referentieniveau, en dus het percentage van de tijd dat de oester is ondergedompeld, blijkt de voornaamste factor te zijn die de lengte van de oester beïnvloedt. Deze relatie wordt echter verborgen door de invloed van massale oestergroei en de nabijheid van de bodem.

In het tweede gedeelte hebben we de hypothese getest dat de vorm van de oester wordt beïnvloed door ecologische factoren. In een eerste benadering heben we de oesterschelpen beschreven door middel van éénwaardige vormvariabelen. Deze werden gecorreleerd (rang correlatie) met tal van omgevingsvariabelen. De vormvariabelen die de onregelmatigheden van de schelp rand het best beschrijven zijn sterk gecorreleerd (rang correlatie) met de hoogte boven het referentieniveau. In een tweede benadering wordt een beeldanalytische techniek die de omtrekken van de 85 verzamelde oesters determineert beschreven. Deze omtrekken worden numeriek gekarakteriseerd door middel van een elliptische Fourier analyse. Omdat de Fourier coëfficiënten kunnen worden genormaliseerd, zodat ze onafhankelijk worden van veranderingen in grootte en rotatie, evenals van andere transformaties van het oorspronkelijke beeld, kunnen ze worden gebruikt als variabelen in een multivariate analyse van vormen. Clusteranalyse onderscheidt verschillende vormen van de mangroveoester *S. cucullata* . De vertikale distributie, de vorm van het substraat en de oriëntatie ten opzichte van de getijdestroming blijken factoren te zijn die gecorreleerd zijn met de vorm van *S. cucullata.*

Hoofdstuk 3: Het mangrove-ecosysteem

In dit hoofdstuk wordt nagegaan in welke mate grondwaterstroming een effect heeft op de distributie van het mangrovebos.
Mangroves zijn de enige bescherming in tropische regio's tegen kusterosie. Ze zijn tevens kraamkamers voor een groot aantal dieren en bovendien zorgen ze voor een versnelde sedimentatie van partikels die naar de kust worden gespoeld door rivieren. De distributie van mangroves in tropische en subtropische regio's is meestal gebonden aan de aanwezigheid van estuaria of kreken. Algemeen bestaat er een consensus dat het brakke water waarin het mangrove-ecosysteem voorkomt, zijn verlaagde saliniteit verkrijgt door de aanvoer van zoet water door rivieren. Nochtans vinden we over de gehele wereld verspreid voorbeelden van mangrovegebieden zonder merkbare toevoer van zoet water. Door gebruik te maken van een grondwatermodel wordt in een eerste deel van dit hoofdstuk aangetoond dat mangroves slechts voorkomen in gebieden met een minimum grondwaterstroming van $1 m^2$ per dag. Het model toont tevens aan dat het oppompen van drinkwater op grote afstand van de kust op termijn kan leiden tot de vernietiging van grote man-

grovegebieden. Het gebruikte model voorspelt of bevestigt de vernietiging van mangrovegebieden in Kenya en in Florida (USA).
In een tweede deel van dit hoofdstuk worden de effecten van de grondwaterstroming langsheen de Keniase kust bestudeerd in funktie van de relatieve frequentie, de gemiddelde maximale boomhoogte, de biomassa per m^2 van het bovengrondse deel van de boom en het aantal propagules per m^2 van 2 mangroveboomsoorten: *Avicennia marina* en *Rhizophora mucronata*. Omwille van zijn dubbele zonatie werd *A. marina* afzonderlijk bestudeerd in de land- en zeewaartse zone van het mangrovebos. Hoge grondwaterstroming bevoordeelt de concurrenten van *A. marina* voor wat betreft de kolonisatie van de kustzone. Hogere grondwaterstroming gaat gepaard met mangrovebomen met een grotere gemiddelde maximale hoogte en een hogere biomassa voor het boomgedeelte boven de grond. Het aantal propa-gules per m^2 daalt bij een toenemende grondwaterstroming. Dit is vermoedelijk een resultaat van de hogere overlevingskansen van de propagules in regio's met een lagere saliniteit. *A. marina*, groeiend aan de landzijde van het mangrovewoud, vertoont een aantal aanpassingen aan de drogere, meer zout bevattende bodem.

Hoofdstuk 4: Voedingsgedrag

Een aantal kustorganismen, waaronder bivalven, staan gekend als belangrijke verbruikers van particulair organisch koolstof (POC) in de zee. In een eerste deel van dit hoofdstuk geven we nieuwe bewijzen voor het feit dat deze organismen ook belangrijke verbruikers zijn van opgelost organisch koolstof (DOC) van colloïdale oorsprong (diameter > 0.2 µm). Deze colloïden spelen een belangrijke rol in de globale flux van koolstof in de zee. In dit deel vergelijken we de opname van colloïdaal DOC bij mariene bivalven voorkomend in de Noordzee en in het westelijk deel van de Indische Oceaan. Om deze opname te kunnen meten hebben we gebruik gemaakt van colloïdaal melanine.
In een tweede deel van dit hoofdstuk werd het voedingsgedrag van *S. cucullata* bestudeerd. *S. cucullata* neemt grote hoeveelheden opgelost organisch koolstof (grootteorde 0.2 tot 1.2 µm) op tijdens de eerste twee uren dat de oester ondergedompeld is. Het grootste deel van dit opgelost organisch koolstof bestaat uit colloïdaal materiaal en uit bacteriën. *S. cucullata* vertoont even-

wel een duidelijke voorkeur voor de opname van bacteriën. Omwille van de hoge concentraties aan colloïdaal materiaal is deze voedingsbron even- wel de belangrijkste voor de mangroveoester. Het opgenomen collo-ïdaal materiaal vertoond een $\delta^{13}C$ waarde die vergelijkbaar is met de $\delta^{13}C$ waarde van zeegrassen in het gebied waar de oesters vandaan komen. Tussen het derde en het vierde uur na onderdompeling verschuift de preferentiële opname van *S. cucullata* van DOC naar POC. Naast het zoo- en fytoplankton neemt *S. cucullata* ook grotere sestondeeltjes op. Deze laatste zijn afkomstig van rottende mangrovebladeren. De opname van particulair organisch koolstof vertoont een sterke relatie met de diameter van deze deeltjes. Naarmate de tijdsperiode dat *S. cucullata* ondergedompeld is gro-ter wordt, wordt de diameter van de opgenomen deeltjes ook groter.

Hoofdstuk 5: Kolonisatiegedrag

In dit hoofdstuk wordt het kolonisatiegedrag van *S. cucullata* larven bestudeerd. Dit hoofdstuk bestaat uit drie grote delen.

In een eerste deel worden de kweek van *S. cucullata* larven beschreven tot op het ogenblik dat ze zich vasthechten op hun substraat. Duidelijke verschillen in het aantal bevruchte eicellen per oester werden waargenomen in funktie van de zoutconcentratie van het zeewater. Hoe lager de saliniteit, hoe groter het aantal bevruchte eicellen per oester. Wanneer de larven worden gevoed met een combinatie van *Chaetoceros sp.* en *Nitzschia sp.* neemt de larvale periode, van bevruchte eicel tot metamorfose, ongeveer 28 dagen in beslag. Vierentwintig uur na de bevruchting vertonen de larven de neiging om te zinken. Deze neiging wijzigt zich in een stijging onder invloed van een dalende saliniteit. Vooral op het einde van de larvale periode is dit verschijnsel zeer duidelijk.
In een tweede deel werden volwassen mangroveoesters gescheiden van hun 'biofilms' als mogelijke bron van wateroplosbare chemische stoffen die het kolonisatiegedrag van *S. cucullata* induceren. Hiertoe werd het vertikale en horizontale zwemgedrag van de larven bestudeerd gebruik makende van beeldanalyse. De studie beschreven in dit hoofdstuk toont voor de eerste maal een opeenvolgende reeks van chemische signalen aan die uiteindelijk leiden tot het vrijkomen van een chemische stof die in een voldoende hoge

concentratie de oesterlarven aanzet om zich vast te hechten op het dichtstbijzijnde substraat.
Een derde deel in dit hoofdstuk behandelt de voordelen van het vormen van aggregaten van volwassen oesters. In dit deel wordt aangetoond dat dergelijke aggregaten een positief effect hebben op het kolonisatiegedrag van *S. cucullata* larven en op de overleving van deze larven.

Hoofdstuk 6: Epiloog

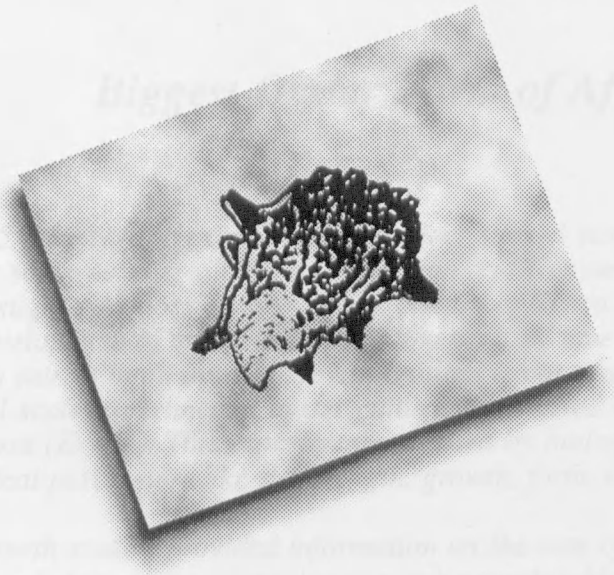
In de epiloog worden de resultaten van de verschillende artikels aan elkaar gekoppeld. We vatten hier kort de conclusies samen die betrekking hebben op de distributie van *S. cucullata*.
Betreffende de 'macro'-distributie van *S. cucullata* komen we tot volgende besluiten:

- mangrovebomen vormen het belangrijkste substraat beschikbaar voor *S. cucullata*;
- grondwaterstroming beïnvloedt zowel de distributie van het mangrovebos als het aantal bevruchte eicellen voortgebracht door volwassen exemplaren van *S. cucullata*; en
- detritus afkomstig van mangrovebomen is een bron van voeding voor *S. cucullata*. Deze bron wordt belangrijker naarmate *S. cucullata* langer ondergedompeld blijft.

Betreffende de 'micro'-distributie van *S. cucullata* komen we tot volgende besluiten:

- het aantal bevruchte eicellen per volwassen *S. cucullata* exemplaar verhoogt in funktie van een dalende saliniteit;
- het zich vastzetten van *S. cucullata* larven in de buurt van aggregaties volwassen oesters is het gevolg van een opeenvolgende reeks van chemische signalen die uiteindelijk leiden tot het vrijkomen van een chemische stof die in een voldoende hoge concentratie de oesterlarven aanzet om zich vast te hechten op het dichtstbijzijnde substraat;
- het zich vastzetten van *S. cucullata* larven in de buurt van grote groepen volwassen exemplaren leidt tot grote aggregaten van deze soort; en
- aggregaties van *S. cucullata* kunnen grote hoeveelheden oesterlar-

ven aantrekken en verhogen de overlevingskans van deze larven die zich in de onmiddellijke omgeving van de aggregaties hebben vastgehecht.



APPENDIX

On March 29, 1993 the following article was published in 'DAILY NATION', one of the major newspapers in Kenya:

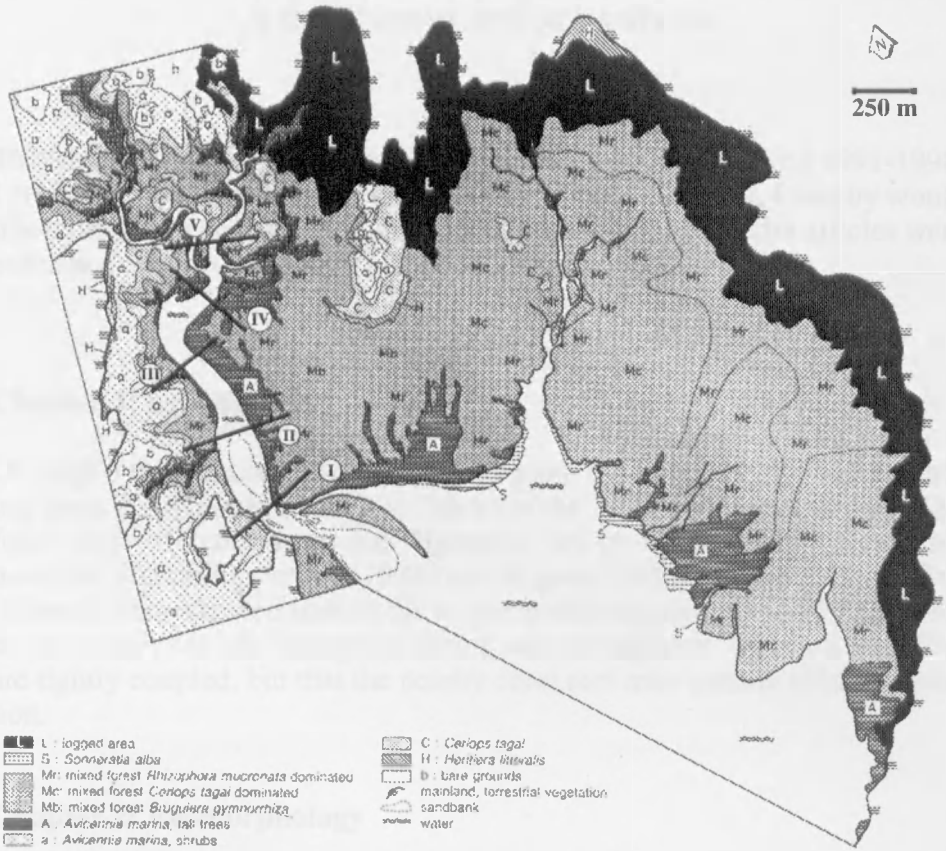
Biggest Oyster Farm of Africa

In 1985, when the Kenya Belgium Project started research in the field of marine sciences, a small group of Belgian and Kenyan researchers started to investigate the possibility of oyster culture in Kenya.
The development of an oyster culture takes a long time especially when you want to start it in an area where information is not available.
A small-scale experimental oyster culture was started on the south coast of Mombasa (Kenya). This experiment was used by biologists to study various biological parameters like shell length, growth, form, weight, breeding, and so on.
The growth studies provided information on the rate of growth - indicating the period of time the oyster takes to reach a marketable size, the times of the year for the most rapid growth, the variation in growth over the study area and the differences in growth with tidal level or with depth below the surface.
Once this information was known, a second phase in the research started. This was on the breeding cycle. This study was necessary to determine when spatfall occurred, the sites with the best spatfall and the most desirable depths or levels for spatfall.
In the experiment, the usefulness of local materials as spat collectors was studied. Ropes, rocks, coconut shells and tiles were tested to their usefulness as spat collectors in the mangroves.
Collection of spat, based on breeding information, is the foundation on which any oyster culture rests.
The researchers working under the Kenya/Belgium Project in Marine Sciences were also doing research in other fields associated with oyster culture.
Among these is the productivity of the mangrove waters as related to nutrient levels and to the various components of the plankton, generally regard-

ed as oyster food.
After several years of testing a method was developed to culture the oysters in such a way that they reach maximum size in the shortest time possible. The method used here was already used in the Netherlands although some modifications were made to suit the local conditions.
Several economic experts visited the project to assess the possibility of commercialising this kind of culture. Their reports were positive.
In the last 10 years, the number of oysters consumed by tourists in the restaurants along the Kenya coast increased drastically. An estimated 25 million oysters were consumed in 1990. Tamarind Restaurant alone used six hundred thousand oysters for their restaurants in Mombasa and Nairobi.
While the consumption of the oysters is increasing every year the size of the oyster is decreasing every year.
To be able to offer to the tourist industry large sized oysters and at the same time stop the destruction of mangrove forests, the Government through Kenya
Marine and Fisheries Research Institute asked the Belgian Government to send an expert on oyster culture to commercialise it here.
Since October 1991 Mr. Tack, a Belgian working under the Kenya/ Belgium Project at KEMFRI, started the building of the first commercial oyster culture in Eastern Africa. To date this is the only commercial oyster in East-Africa and the biggest on the African continent.

In the meantime a second commercial oyster culture was built in Shirazi, 65 km south of Mombasa, with the financial aid of the Co-operation section of the Belgian Embassy in Nairobi. This oyster culture is owned and managed by a local women's group.

Gazi (Kenya): map



Vegetation Map of Gazi Bay. Map drawn on the basis of aerial photography and ground observations (after F.J. Slim *et al.*, 1996).

References

Slim, F. J., Gwada, P. M., Kodjo, M., Hemminga, M. A. (1996). Biomass and Litterfall of *Ceriops tagal* and *Rhizophora mucronata* in the Mangrove Forest of Gazi Bay, Kenya. Mar. Freshw. Res. 47: 999-1007

Additional information

This study consists of a number of articles written in the period 1991-1998. I preferred to use the original articles in this study. However, I hereby would like to include a number of comments made by colleagues after articles were published or went in press.

Chapter 1: Introduction

On page 6 we mention the existence of a large oyster bed on the reef extending from Ras Ngomeni. Even in this case the mangrove forest is near. The fossil reef extending from Ras Ngomeni lies in the middle of a seagrass meadow. Hemminga *et al.* (1994) investigated carbon fluxes in Gazi Bay (Kenya). They showed that, as far as particulate organic matter (POM) fluxes are concerned, the mangrove forest and the adjacent seagrass meadows are tightly coupled, but that the nearby coral reef may exist in relative isolation.

Chapter 2: Ecomorphology

On page 19 and 20 we describe the cluster analysis used. Ward's method was used for clustering. The distance measure used is the squared Euclidean distance. There exists other methods and other distance measures. Most of them showed similar results. The specific choice of Ward's method and the squared Euclidean distance was based on a similar choice in Vanden Berghe (in press).

Page 22, Figure 2. Both a and b are used as an indicator of orientation of the shell towards the tidal current: a stands perpendicular to the tidal current on

the side of the stem or stilt root facing the incoming tide, while b stands perpendicular to the tidal current on the side of the stem or stilt root facing the outgoing tide.

Chapter 3: The Mangrove Ecosystem

On page 64 the basin characteristics that serve as input for the model are described: transmissivity values T, areal net precipitation R, topographic levels h_t, aquifer thickness d and porosity of the aquifer material n. We have to mention that we made use of mean values over large areas. There are regional differences in those values. Sometimes those differences can be quite large (e.g. topographical levels along the coast can change with almost 100 m over a distance of 10 km). Those differences will influence the real groundwater outflow. When we interpret the outcome of the model we have to keep in mind those input restrictions.

Chapter 4: Feeding Behaviour

On page 102 and 103 we describe the methodology used to compare the uptake of colloidal melanin from seawater by marine bivalves from tropical and non-tropical region. It is important to mention that the species used were studied in their own environment. Tropical species were studied at a temperature of 27°C, while North Sea species were studied at a temperature of 12°C.

On page 106 we discuss two articles by Suzuki et al. (1985) and Sugimura and Suzuki (1988). Today we know that the methodology they used was incorrect. At the time the article 'The uptake of Colloidal Melanin from Seawater by Marine Bivalves' was accepted by Marine Ecology this information was not available.

References

Hemminga, M.A., F.J. Slim, J. Kazungu, G.M. Ganssen, J. Nieuwenhuize &

N.M. Kruyt, 1994. Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). Mar. Ecol. Prog. Ser., **106**: 291-301.

Vanden Berghe E., in press. The Form of Oysters in Relation to the Form of the Substrate. In: "Symposium Proceedings of the HYSEA Annual Symposium on State of Knowledge and Recent Research Advances in Freshwater and Marine Biology in Eastern Africa".