# A TAXONOMIC, ECOLOGICAL AND COMMERCIAL POTENTIAL STUDY OF THE GENUS *GRACILARIA* (GRACILARIALES, RHODOPHYTA) OF THE KENYA COAST.

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

---------- Date 7/12/93

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This thesis has been submitted for examination with my approval as the University Surpervisor

---------- Date 7/12/93

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Professor of Botany.

## DEDICATION

This work is dedicated to

Joe

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## ABSTRACT

A study was carried out on the taxonomy, ecology and the yield as well as properties of the polysaccharides of the marine algal genus Gracilaria, which is one of the genera of commercial importance worldwide. Eight species of the genus were investigated and reported with respect to their morphological and anatomical characteristics. The species are: G. corticata, G. crassa, G. edulis, G. fergusonii, G. millardetii, G. salicornia, G. verrucosa and Gracilaria sp. Two species, G. corticata and G. salicornia were observed to be highly variable morphologically and their variability depended on the prevailing physiological and ecological conditions. As taxonomic features, the branching pattern and whether the thallus is terete or flattened was shown to be quite consistent while the other features were observed to exhibit a lot of variations. Due to morphological plasticity previous workers reported a total of thirteen species for the Kenyan taxa and it has now been established that some of these are but synonyms.

With respect to their distribution along the Kenya coast, G. salicornia was observed to be the most common followed by G. corticata and then G. crassa. The rest would be classified as not common. The north-south distribution of the species did not show any specific trend though stations with rocky platforms had a wider variety of species than the sandy and mangrove beaches. Most of the

species grew on rough substrate in the eulittoral zone with a higher percentage in the lower eulittoral. Only G. salicornia was observed to extend from the upper eulittoral to the sublittoral in its distribution while G. verrucosa was basically sublittoral. One feature that was found to be common to all the eulittoral plants, with an exception of one ecotype of G. salicornia, is that they were either growing in pools or lagoons and as such they were not exposed to stress due to desiccation during low tide.

Studies on the seasonality pattern of the genus showed that seasonal abundance of different species varied with time and geographical location. Nonetheless, a general single peak for the genus was evident between the months of September and December. G. verrucosa, however, was observed to have its peak biomass in July/August and it was also observed to spend part of its life cycle buried in sand. Implications of how environmental factors contribute to the seasonality pattern of the plants are discussed.

Based on their availability in adequate quantities six species were tested for their agar yield and quality. The species tested were G. crassa, G. corticata, G. millardetii, G. salicornia, G. verrucosa and Gracilaria sp. Agar was extracted from as many different ecotypes as there were, covering the major seasons when the plants were available. Chemical and physical properties of the extracts were

studied and comparisons made. Native agar yield from hot water extract was observed to range from 8.1%-30.3% of dry weight with G. verrucosa and G. salicornia yielding the highest and the lowest amounts respectively. Gel strengths of 1.5% solution of the native extracts was observed to be highest in G. verrucosa (220gm/cm$^2$) and lowest in G. corticata (<60gm/cm$^2$) whereas the highest gelling temperature was recorded from Gracilaria sp. extract (40.4°C) and the lowest from G. verrucosa extract (28.9°C). The melting temperature, on the other hand, was highest in agar from G. crassa (94.8°C) and lowest in that of G. corticata (79.8°C).

On determining the concentration of 3,6-anhydrogalactose in the different species it was found that G. verrucosa agar had the highest content (23%) while G. corticata had the least (14.5%). The sulphate content was highest in agar from G. corticata and lowest in that of Gracilaria sp. Treatment of either the plant or native agar extract with alkali (NaOH) only improved the chemical and physical quality of agar from G. crassa, G. salicornia, G. verrucosa and Gracilaria sp. while agar from G. corticata and G. millardetii did not improve much. The yield, physical and chemical properties of these extracts were observed to vary not only from species to species but also from one ecotype to the other and from season to season. When the observations made in this study were compared with those of a commercial agar Difco Bacto Agar, it was established that G. verrucosa yielded agar that was closest to it in terms of their properties.

Implications of the results obtained in this study are discussed with respect to seaweed farming. G. verrucosa, G. crassa and some ecotypes of G. salicornia have been recommended for this. Further investigations with a view to culturing them in mass would be a worthy course.

# CHAPTER 1

# INTRODUCTION

## 1.1. The Algae

The term algae is difficult to define simply because the plants share their more obvious characteristics with other plants while their real unique features are less obvious. Using the aquatic habitat as a distinctive feature for the algae would not suffice since there are a number of liverworts, mosses, ferns and angiosperms that are also aquatic. Moreover, there are also quite a number of terrestrial algae. To distinguish algae from other chlorophyllous plants one has to resort to characteristics in their sexual reproductive systems. These differ from those of other green plants in the following ways:

(a) the organisms themselves function as gametes in cases of unicellular algae,

(b) gametes may be produced in special unicellular containers (gametangium) in some multicellular algae while,

(c) in others the gametangia are multicellular, and every gametangial cell is fertile thus producing a gamete. These characteristics are not known to occur in liverworts, mosses and vascular plants. Most of their multicellular sex organs are only partially fertile, being

covered by sterile cells. Asexual reproduction in many algae is by flagellate spores and/or non motile spores in unicellular sporangia, or if they are multicellular every cell is fertile. (Bold and Wynne, 1985).

Algae can be divided into planktonic or benthic depending on their growth habitat. The planktonic ones are microscopic, usually unicellular and are nomadic, floating freely in the water mass. The benthic group encompasses the larger type which grow attached to rocks or other solid objects in the bottom of the water. Some members of the benthic group are more highly differentiated with rootlike, stemlike and leaflike organs, which characteristically are lacking the vascular tissue. A few algae are neustonic, that is, they live at the interface of water and the atmosphere. The current study focuses on a genus belonging to the marine benthic group, which are also known as "seaweeds".

### 1.2. The Value of Marine Algae

"The American layman's usual experiences with marine algae is in accord with the implications of the word "seaweeds", as a useless or even noxious plant of the sea. Seaweeds may have appeared unsightly to him, littering a lovely beach; they may have entangled his fishing line,

they may have fouled the bottom of his boat or even caused him swimmer's itch. More than likely, he does not realise that he uses seaweed products virtually every day of his life and that our marine algae are an enormous natural resource that has in recent decades become fundamental to numerous industries". (Dawson 1966).

Seaweeds serve important ecological roles such as primary production, nutrient storage and cycling, sediment formation and provision of habitats that support enhanced secondary production (Lapointe, 1989). In the Kenyan coastal waters these roles are served by a rich flora of over 300 species (Moorjani, 1977). Apart from the ecological role that they play, numerous seaweeds have products that are of commercial value. Polysaccharides which are derived from brown and red seaweeds and are able to form colloidal systems when dispersed in water are known as phycocolloids (Tseng, 1945). The industrially important phycocolloids are agar, algin, carrageenan and furcellaran and of these agar has the highest value. In addition to the phycocolloids other products such as iodine are also derived from seaweeds. Marine algae have also been known to be useful as food for man and domestic animals, compost manure, fish bait and even medicine. The use of seaweed as a source for colloids, especially agar, is the aspect that this

study concerns itself with. The colloids have unique gelling properties that determine their application in different industries.

Both the industrialized and the developing countries make use of the phycocolloids from marine algae in very many different ways. Since the countries of the latter category are bound to continue establishing more industries of their own as they develop, their imports of the phycocolloids are bound to increase. There is, therefore, every reason to believe that the demand for these products will continue to be high in the future, hence, the search for new resources is quite appropriate.

Although the Kenyan shoreline supports many agar-bearing seaweeds (Gelidiella, Gelidium, Pterocladia and Gracilaria,) none of these is being exploited commercially either for manufacturing agar locally or for export as raw material. It follows therefore that Kenya has a virgin resource which needs to be explored. Gracilaria is one of the seaweeds that has been commonly reported growing in the Kenyan coastal waters and which is a likely candidate for commercial exploitation. But before one ventures into this, one needs to know those species that produce quality agar in economic quantities, and this information can only be available after screening all the Gracilaria that grow in the Kenyan

waters. Moreover, the production of agarophytes from natural stocks is influenced both by seasonal factors and by harvest pressures exerted on them during the preceding harvesting season (Kain and Norton, 1990). Since their growth cycles are influenced by environmental conditions and by man's exploitive activities they are prone to over-exploitation. The need to manage and conserve the stocks is therefore, of prime importance in order to enhance their productivity and prevent over-exploitation which is always the case in a "free-for-all" situation. It is therefore necessary that the commercial exploitation of natural stocks or any resource be preceded by biological studies which shall be the basis for their management. Knowledge of the reproduction, growth cycles, growth rates, regeneration capacities, productivity and the influence of environmental factors on biomass production is therefore absolutely necessary. Only then can one know where the species is found in abundance, how much can be harvested and when, and how many times a stock can be harvested during a season.

### 1.3. Literature Review

The first published records on some of the Kenyan marine algae can be traced back to Hauck (1886, 1887, 1888, 1889). His publications were taxonomic lists of algae collected by J.M. Hildebrandt

from the Red Sea and the Indian Ocean, Mombasa being one of the localities in the Indian Ocean. Among the taxa published by Hauck are species of Gracilaria. Schmitz (1895) also contributed quite substantively to the knowledge of marine red algal resources of the East African region, especially those occurring in Tanzania and Kenya. He listed 68 taxa, which include species of the tropical genera Gracilaria and Eucheuma which are now very important commercial sources of the colloids agar and carrageenan respectively. Other floristic lists that date up to 1912 were published by botanists who called on the East African coastal stations such as Mombasa, Tanga, Lindi and Dar-es-Salaam. These include Engler (1895), Reinbold (1907) and Schroder (1912). The first paper which deals specifically with the Kenyan algal flora dates back to Gerloff (1960). He examined herbarium collection of Kenyan algal flora made by Greenway and Rawlins, between 1952 - 1953 and 1955 - 1957. This paper is the first publication that includes some ecological observations and the geographical distribution of a single species of Cyanophyta and 35 species of chlorophytes. Thereafter, additional records by authors such as Isaac (1967, 1968, 197l);Isaac and Isaac (1968); Knutzen and Jaasund (1979) have also added significant information to the present knowledge of the species of marine algae occurring on Kenyan shoreline.

So far the records listed above have heavily dwelt on the taxonomy and species lists of the marine algae. Very little work has been done on the ecology of the Kenyan marine algae (Lawson, 1969, Moorjani, 1977; 1980, Oyieke and Ruwa, 1987; Coppejans and Gallin, 1989). Moorjani (1977) in her work carried out a study on the ecology of about 300 species of marine algae and the number could not have allowed a detailed study on each species. She reported a total of 11 species of Gracilaria on the Kenya coast and some of these had been reported by earlier authors. To date only one researcher, Imbamba (1972), has done studies relating to the biochemistry of some of the Kenyan marine algae. He analysed the mineral elements of 6 chlorophytes, 4 phaeophytes, 4 rhodophytes and the marine angiosperm Cymodocea ciliata collected from different stations along the Kenya coast. The elements analysed were nitrogen, phosphorus, potassium, calcium and magnesium. He compared the variations of these elements in the different plants. Knowledge concerning the potential of commercially important seaweeds in Kenya is very scanty (Ruwa, 1981; Yarish and Wamukoya, 1990) and are based on field surveys.

Among the taxa reported by the above authors are species of Gracilaria. However, there is no published record on their ecology,

distribution and biochemical studies on their phycocolloids. As mentioned earlier Gracilaria species are some of the potential agar sources that are being tapped worldwide. This, therefore, necessitates a study on the genus to assess gels from different species with a view of determining the ones with properties suitable for industrial utilization and which would be appropriate for commercial cultivation eventually.

### 1.3.1. The Genus Gracilaria Greville

Gracilaria belongs to the family Gracilariaceae and it was considered as a member of the order Gigartinales until 1989 when Fredericq and Hommersand (1989) proposed the order Gracilariales based on the reproductive development of Gracilaria verrucosa. The family is a member of subclass Florideae and division Rhodophyta. Family Gracilariaceae currently contains seven genera: Gracilaria, Gracilariopsis, Gracilariophila, Hydropuntia, Curdiea, Melanthalia and Congracilaria. All members of the family are characterised by the following features of the female reproductive systems (Fredericq and Hommersand, 1989):

(1) a supporting cell of intercalary origin that bears a two-celled carpogonial branch flanked by two or more sterile branches

(2) direct fusion of cells of the sterile branches onto the persistent carpogonium

(3) isolation of the carpogonium upon degeneration of the hypogynous cell leading to the formation of a generative fusion cell that cuts off several gonimoblast initials

(4) formation of an ostiolate pericarp

(5) schizogenous development of the cystocarp cavity

(6) secondary fusion of gametophytic tissues

Diagnostic characters that separate genera include;

(1) the extent of incorporation of additional vegetative cells into the fusion

(2) the presence and nature of any nutritive tissues

(3) the specific character of any secondary fusions and

(4) the mode of gonimoblast maturation

Following is a key, according to Fredericq and Hommersand (1990), to members of the genus:

1. Parasitic on other Gracilariaceae; hemispherical pustule.....2

1. Free living of various forms .....3

2. Cystocarp lacking tubular nutritive cells linking

gonimoblast and pericarp, spermatangia superficial

..... Gracilariophila

2. Cystocarp with tubular nutritive cells linking gonimoblast and pericarp; spermatangia, organized in 'conceptacles'

.....Congracilaria

3. Cystocarp cavity not completely filled by gonimoblast; tubular nutritive cells present or absent, cruciate tetrasporangia superficial, scattered in outer cortex.

..... 4

3. Cystocarp cavity completely filled by gonimoblast; tubular nutritive cells absent, carposporangia in long files, cruciate tetrasporangia between raised cortical filaments (nemathecia) .....6.

4. Tubular nutritive cells absent in cystocarp: spermatangia superficial .....Gracilariopsis

4. Tubular nutritive cells absent in cystocarp; spermatangia organized in "pits" or 'conceptables .....5

5. Tubular nutritive cells present in both pericarp and floor of cystocarp; spermatangial conceptacles generally not

confluent.....Gracilaria

5. Tubular nutritive cells restricted to floor of cystocarp; spermatangial conceptacles commonly confluent.
.....Hydropuntia

6. Branching pseudodichotomous; branch apices with thickened margins; cystocarp with thick-walled sterile gonimoblast tissue
.....Melanthalia

6. Branching variable, not pseudodichotomous; branch apices without thickened margins; cystocarp lacking thick walled sterile gonimoblast tissue .....Curdiea

The genus has some 100 species described in the subtidal and intertidal zones of temperate, tropical and antarctic regions (Santelices and Doty, 1989; Yamada, 1976). The majority of species occur in warm waters and tropical habitats and they are considered to have evolved in warm waters, although the greatest standing stocks are known from temperate regions. An analysis of the distribution of eastern Pacific and Western Atlantic species, has shown that most species are limited to regions where water temperatures are 20°C or higher for at least three months of the year (McLachlan and Bird, 1986).

Members of the genus Gracilaria are of moderate size, erect or form cushions. They are freely branched and they possess external sessile, hemispherical or beaked cystocarp with a thick pericarp. A single ostiole and ellipsoidal spores arise from a large parenchymatous placenta. Spermatangia occur in superficial sori or in conceptacle-like depressions in the thallus surface. Tetraspores are cruciate and are scattered on the periphery of the thallus. The thallus is parenchymatous and consists of large central cells and a varying development of intermediate cortical cells and external layer of small peripheral cells. In development of cystocarp carpogonial filament is two-celled and its supporting cell bears sterile filaments. These cells later fuse with each other and the adjacent cells. From the fusion the gonimoblast filaments grow toward the surface of the thallus and all the cells of this mass, except those at the centre develop into carposporangia. Carpospores are shed beneath the pericarp and liberated via ostiole (Durairatnam, 1961).

Gracilaria reproduces both vegetatively and by sporulation and it exhibits Polysiphonia-type life history (Chen, 1973). Its gametophytes are heterothallic and the male thallus produces spermatangia. The eggs produced by the female gametophyte are fertilized and develop into zygotes within the female gametophyte thus giving rise to a diploid carposporophyte phase. When carpospores are released they germinate

into free living diploid tetrasporophytes. Tetraspores are produced from the tetrasporophyte and germinate into male and female gametophytes. The life history is summarised in figure 1. Mathieson (1975) reports that a single Gracilaria plant produces over 60,000 tetraspores and more than 40,000 carpospores. Studies done by Bird et al. (1977) on the life history of Gracilaria in vitro revealed a male to female ratio of 1:1 from plants originating from tetraspores and that only female plants grown in the presence of male plants formed fertile cystocarps. They further demonstrated that detached plants showed the greatest reproductive potential. Their reproductive maturation occurred in 8-10 weeks after spore germination.

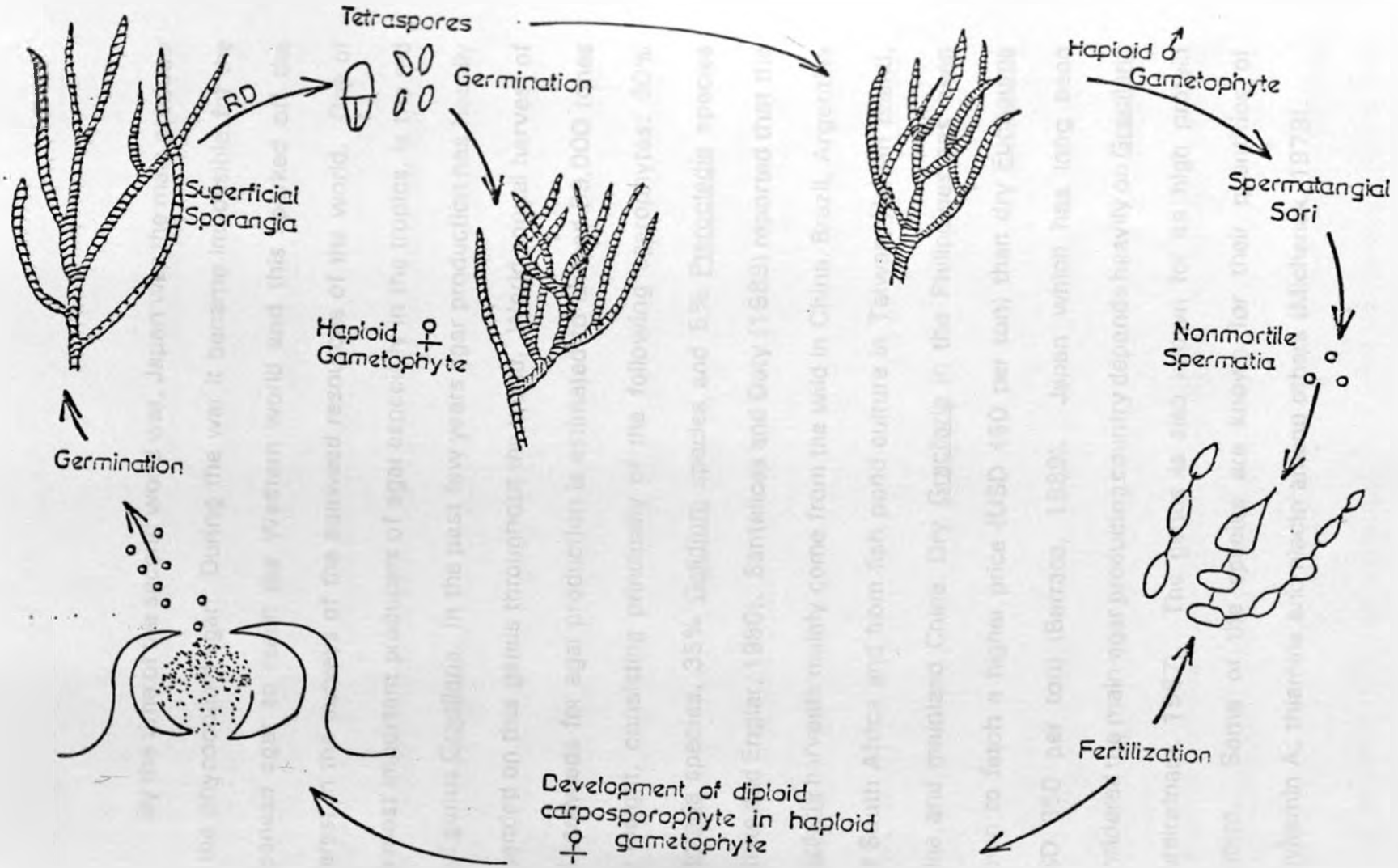



Fig 1. Diagramatic life history of Gracilaria (after Dawson 1966)

By the time of the second world war, Japan was the main producer of the phycocolloid agar. During the war it became impossible for the Japanese agar to reach the Western world and this sparked off the interest in the surveys of the seaweed resources of the world. One of the most important producers of agar especially in the tropics, is the red algal genus Gracilaria. In the past few years agar production has heavily depended on this genus throughout the world. World annual harvest of red seaweeds for agar production is estimated to exceed 39,000 tones dry weight, consisting principally of the following agarophytes: 60% Gracilaria species, 35% Gelidium species and 5% Pterocladia species (Whyte and Englar, 1980). Santelices and Doty (1989) reported that the Gracilaria harvests mainly come from the wild in China, Brazil, Argentina and South Africa and from fish pond culture in Taiwan, Hainan Island, China and mainland China. Dry Gracilaria in the Philippines has been shown to fetch a higher price (USD 450 per ton) than dry Eucheuma (USD 350 per ton) (Barraca, 1989). Japan which has long been considered the main-agar producing country depends heavily on Gracilaria (Durairatnam, 1987). The genus is also known for its high protein content. Some of the species are known for their quantities of provitamin A, thiamine and niacin among others (Michanek, 1979).

Due to the increase in the industrial demand for agar a lot of interest has been taken into the investigations of the biology of the genus (Edelstein et al. 1976 ; Mshigeni and Weevers, 1979; Bird et al. 1977; Dong Ho Kim, 1970; Lindsay and Saunders, 1977a & b; Chapman et al. 1977). Umamaheswara (1972) also did an extensive ecological study on the Gracilariacea of the seas around India. A lot of effort has been put into taxonomical studies of the genus (Abott and Norris, 1985; Chapman et. al. 1977; Bird and Mclachlan, 1984; Umamaheswara, 1972). Taxonomy at the molecular biological level by comparison of genomes is becoming increasingly popular among systematists and it has been established that the chromosome number in Gracilaria is n = 24 (Bird et al. 1989; Cheney, 1988; Yamamoto, 1978). Sexual hybridization studies are also being carried out for taxonomic purposes as well as in the search for better commercial strains (Zhang and Xiugeng, 1989).

Jaasund (1976), in his work on the intertidal seaweeds of Tanzania, listed seven species of Gracilaria.. These are G. corticata, G. millardetii, G. crassa, G. salicornia, G. edulis, G. fergusonii and G. arcuata. Mshigeni (1983) contends that some of the other species that have been reported from other parts of the East African region could be just synonyms of Jaasund's species. He further recommends that of the

species reported by Jaasund in the waters of East Africa, there are only four that appear to be most attractive commercially and these are: G. crassa, G. corticata, G. fergusonii and G. verrucosa.

Overexploitation of stocks of raw material in most countries has led to an extensive search for possibilities of culturing species of the genus in order to supplement natural resources (Lindsay and Saunders, 1979; Yung Shang, 1976; Mathieson, 1975). For a long time cultivation of seaweeds has been practiced in Japan (Boney, 1965; Dawson, 1966 and Kurogi 1963). Cultivation and culture studies have been carried out by Raju and Thomas (1971), Goldstein (1974), Bird (1975), Shang (1976), McLachlan and Edelstein (1977), Lindsay and Saunders (1977a & b), Chiang (1981), Edelstein et al. (1981) and Yoneshigue - Braga and Baeta Neves (1981).

### 1.3.2. The Phycocolloid, Agar

The synthesis and assembly of the cell wall components are the end products of many metabolic processes each of which is the result of the transcription of information from the red algal genome. The cell wall here is considered to include those polymeric materials originating through the metabolic activities of the algae and lying exterior to the

plasmalemmal membrane (Craigie, 1990). The polysaccharides may be grouped according to their presumed biological function as the more rigid structural (ß-linked) galactans such as cellulose, mannans and xylans, and the more flexible, frequently sulphated galactans that comprise the matrix in which the skeletal fibres are embedded. Agar, which belongs to the latter group of polysaccharides, is found in species of red algae known as agarophytes (Tseng, 1945). Originally the Malay term "agar" was meant for a gelling substance extracted from Eucheuma which is now known as carrageenan (Waaland, 1981). These polysaccharides are of high molecular weight and they are also found in the intercellular matrix (Craigie and Leigh, 1978; Mackie and Preston, 1974). Agar is a member of the agar family of colloids which collectively may be termed agarocolloids (Craigie, 1990). The masked agarlike galactans, some of which form precursors for agar may be termed as agaroids.

Agar is basically repeating units of the strongly gelling polysaccharide agarose (Araki,1966; Rees, 1969). Agarose is mainly a poylmer of strictly alternating 3-O-linked ß-D-galactopyranose and 4-O-linked 3,6-anhydro-$\alpha$-L-galactopyranose (Araki, 1966; Araki and Arai, 1957; Hansen et al. 1981; Rees, 1969) (figure 2). Agarose has been defined by Yaphe (1984) as that mixture of agar molecules having the lowest charge

content and therefore, the greatest gelling ability. The structure of agarose may be masked or altered in a number of ways by the substitution of hydroxyl groups with methoxlys or sulphates in various combinations (Rees, 1969; Turvey, 1978). When the 3,6-anhydrous residue is replaced by $\alpha$-L-galactose-6-sulphate, then the product is a chemical precursor of agarose (Rees, 1961).

The properties of the various substituent groups determine the properties of the gel. The agarose macromolecule itself can be heterogenous. The repeating disaccharide structures may be interrupted by sequences of masked repeating units to create block structures within the polymer.

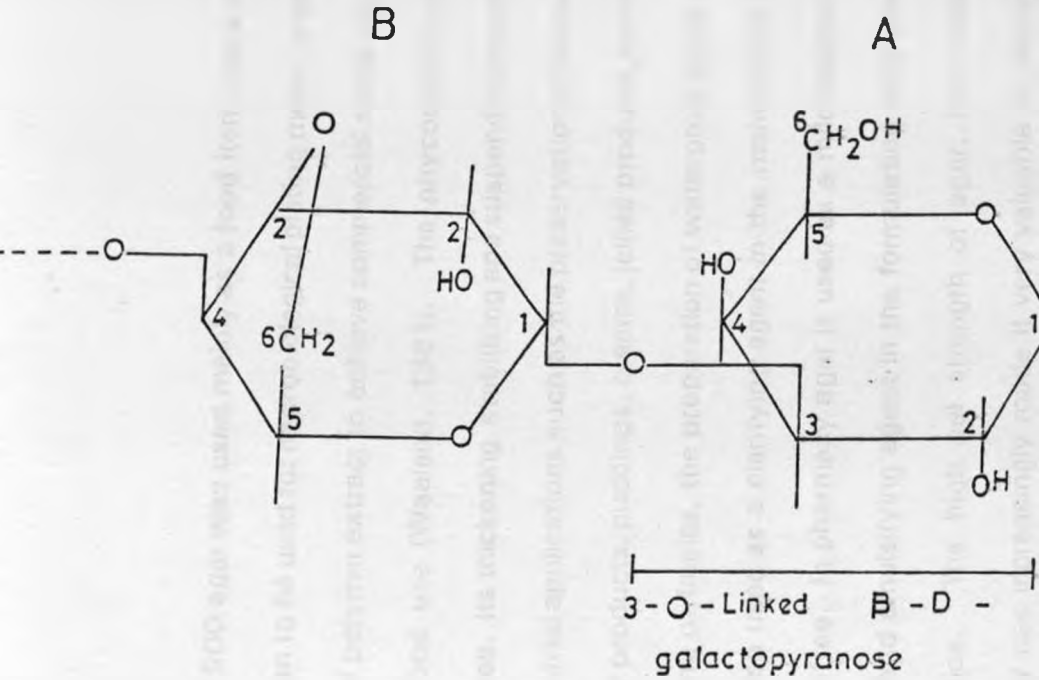
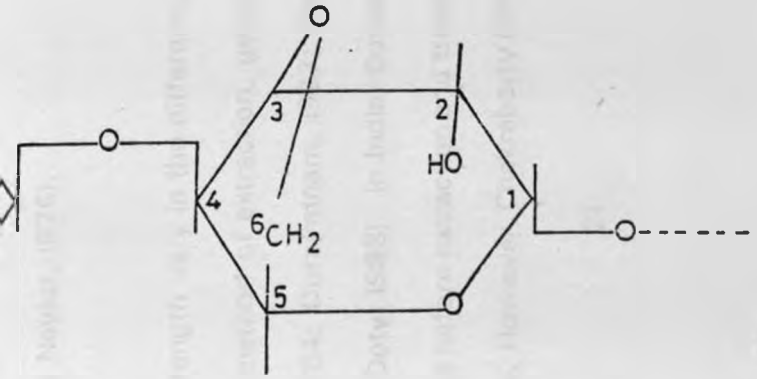



Fig. 2. Disaccharide repeating units of

B




4 - O - Linked 3, 6 - anhydro

$\alpha$- L - galactopyranose

agarose

Until 1900 agar was used mainly as a food item. As a significant quantity began to be used for microbiological plating media, it became the first seaweed polymer extract to achieve commercial status for purposes other than food use (Waaland, 1981). The phycocolloid now has a number of uses. Its thickening stabilizing and suspending properties give it many industrial applications such as the preservation of canned meats, use in bakery products, puddings, creams, jellied products, confectionery making , sizing of fabrics, the preparation of waterproof paper cloth and glue. It is also used as a clarifying agent in the manufacture of wines, beers and coffee. In pharmacy agar is used as a mild laxative and as a suspending and emulsifying agent in the formulation of ointment bases and cosmetics. The high gel strength of agar, low viscosity and transparency has increasingly made it very valuable for microbiological work (Yung Shang, 1976; Naylor, 1976).

Agar yield and gel strength vary in the different species and strains and also depend on the method of extraction, geographic location and season (Craigie et al. 1984; Durairatnam, 1987; Patwary and van der Meer, 1983; Santos and Doty, 1983). In India, Bose et al. (1943) soaked the seaweeds for 18 hours before extraction and treated them with acetic acid to remove impurities. However, Chakraborty (1945) did the same but

used the process of freezing and thawing for purifying the agar gel and used active carbon to decolour the gel. Karunakar et al. (1948) on the other hand purified the gel by soaking in water of low salt content for 96 hours and finally washing the gel under pressure at 23°C, after freezing, the gel was dried with acetone. Thivy (1951) soaked the seaweeds for 24 hours prior to extraction but Kappana and Rao (1963) comparatively studied Thivy's operation and found that soaking and extraction under pressure reduced the quality of the agar gel. Srinivasan and Santhara (1965) washed the sea-weeds in seawater and then in fresh water several times and dried them in the sun until they were completely bleached. Extraction was done by boiling the seaweeds at pH. 6, and the extract was then filtered and frozen. This method was similar to that of Durairatnam and Medcoff (1954) but the method of Durairatnam and Santos (1981) differed from the former in that alkaline treatment was used prior to extraction.

Diaz-Piferrer and de Perez (1964) showed that agar content in different species varied in plants when unbleached, bleached and treated with 1% hydrochloric acid to eliminate carbonates. Tagawa and Kojima (1972) demonstrated that agar isolated from Gracilaria is suitable for commercial purposes only after treatment with alkali. Duckworth et al. (1971) on the

other hand, showed that agars from Gracilaria ferox and G. dominaensis improved very little following alkali treatment.

The chemical and physical properties of the agars produced by Gracilaria and the factors which influence these properties are of primary concern. The seasonal and environmental factors affecting these properties have been investigated by several researchers (C. Bird, et al. 1977; Hoyle, 1978 a, b; De Boer 1979; Bird et al. 198l; Rosenberg and Ramus 1981, 1982a, b; Penniman 1983; Bird 1984; Lapointe 1987). There is an inverse relationship between tissue nitrogen (or protein) and carbon (or carbohydrates) in many seaweeds (Dawes et al. 1974; Lapointe, 198l). However, C. Bird et al. (1977) and Penniman (1983), have reported exceptions to this correlation. Some Gracilaria species may exhibit differences in the absolute content of agar between the reproductive phases (Kim and Henriquez, 1979, Whyte and Englar, 1979; Whyte et al. 198l). On the contrary to this Penniman (1977) suggested that no significant differences in agar yield existed among different reproductive phases of G. tikvahiae (as G. foliifera (Forssk.) Boerg). Similar results have been reported for G. coronopifolia J. Agardh and G. bursapastoris (Gmelin) Silva from Hawai (Hoyle, 1978a and b). The variations in agar quality, even within the same species, has led to several detailed studies

on the chemical structure of the polymer (Asare, 1980; Duckworth and Yaphe, 1971; Lahaye and Yaphe, 1988; 1989; Lahaye et al., 1986).

### 1.4. Aim of the Study

With the foregoing introduction it is therefore the aim of this study to identify suitable Gracilaria species, along with their optimal environmental requirements, that could economically be exploited as raw materials for commercial agar production. To achieve this aim it is paramount that the following objectives are achieved:-

(i) Identify all the Gracilaria species that occur on the Kenya coast.

(ii) Investigate the ecology of each of the species and map their distribution along the coast.

(iii) Study seasonality pattern of each of the species in their natural habitat.

(iv) Investigate the agar content and quality of each species reported.

# CHAPTER 2

# KENYA COAST ENVIRONMENT

The Kenyan coastal environment has been described in depth by Moorjani (1977). However, there are a few facts pertinent to this study which are worth highlighting. Figure 3 shows the regional setting of the Kenya coast.

## 2.1. General Physiography

The Kenyan coastal belt can be divided into three geological provinces which are, landward from the sea; the coastal plain, the foot plateau and the Nyika (Fig 4). These provinces have been reported for Handu-Fundi Isa area north of Malindi by Williams (1962), Kilifi-Mazeras area by Caswell (1956), Malindi area by Thomson (1956) and Mombasa - Kwale area by Caswell (1953). These provinces cover areas actively involved in the evolution of the continental margin from upper Paleozoic to present.

The coastal plain consists mainly of a series of raised beaches ranging in height from sea level to about 61m contour. It widens from a few kilometers in the south to more than 100 km at the mouth of river

Tana. From the coastal plain the ground rises in steps and attains an altitude of between 61m and 137m. The foot plateau has an overlapping blanket of Pleistocene sediments. Behind the foot plateau is the coastal Range which are a series of residual hills.

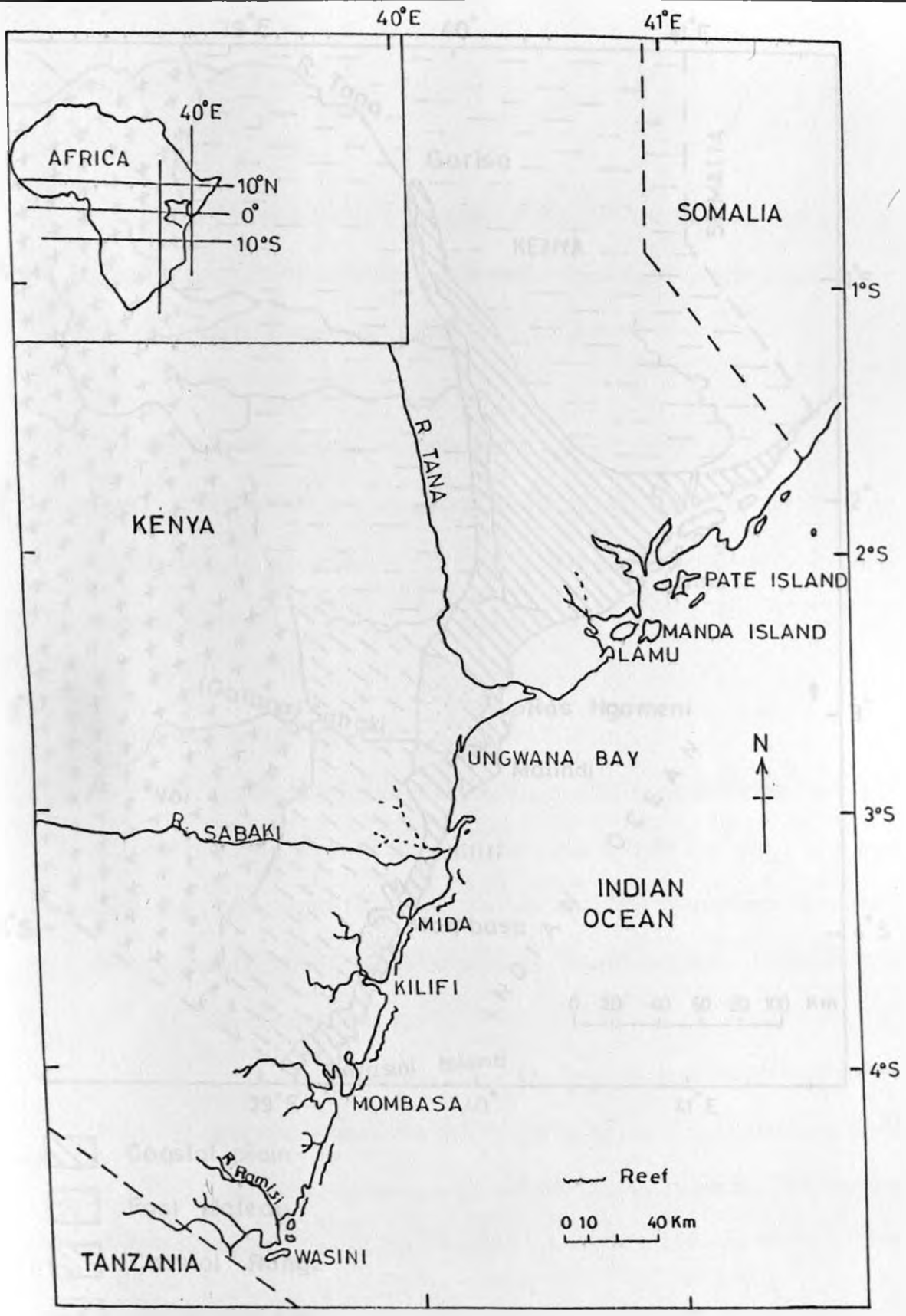



Fig. 3. The Kenya coast. Inset regional location

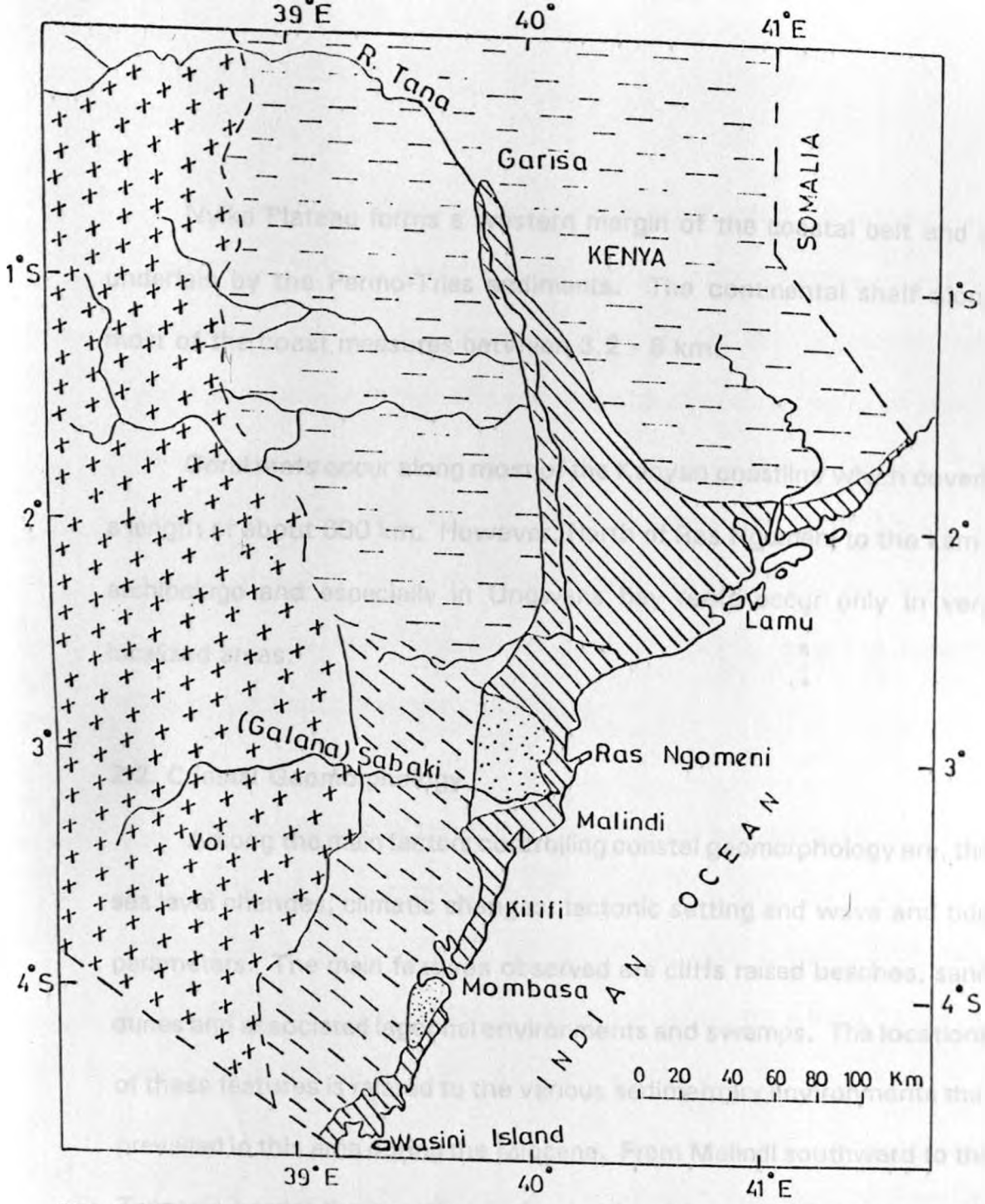



Fig. 4. A general physiography of the Kenya coast.

Nyika Plateau forms a western margin of the coastal belt and is underlain by the Permo-Trias sediments. The continental shelf along most of the coast measures between 3.2 - 8 km.

Coral reefs occur along most of the Kenyan coastline which covers a length of about 600 km. However, North of Ras Ngomeni to the Lamu archipelago and especially in Ungwana bay reefs occur only in very localized areas.

## 2.2. Coastal Geomorphology

Among the main factors controlling coastal geomorphology are, the sea level changes, climatic changes, tectonic setting and wave and tide parameters. The main features observed are cliffs raised beaches, sand dunes and associated lagoonal environments and swamps. The locations of these features is related to the various sedimentary environments that prevailed in this area during the Miocene. From Malindi southward to the Tanzania border the coastline is fronted by either coral limestone reefs that occur as cliffs, beaches and in a few areas swamps. Behind the present shoreline, raised beaches and wave-cut platforms occur parallel to the coast (Lars - Erik 1978).

Cliff bounded shorelines occur in the south coast, Mombasa area, Mtwapa, Kilifi, Watamu and the shoreline from Malindi southward to Kilifi with the exception of the Mida-creek. Observations of rock samples from these areas show that the cliffs are made up of quartz, pieces of corals, numerous shell fragments and microfauna all cemented by calcite. The varied matrix of the rocks has resulted in selective weathering leading to the development of the rough surfaces, overhangs etc. Interspaced with the cliffs are beaches. However, north of Malindi, beaches become predominant especially north of Sabaki.

From Ngomeni northwards the relief of the coastal belt is very low. Seasonal marshes, mangrove swamps and tidal flats dominate. The mangrove swamps occur along the abandoned river lowlands and plains of present rivers. Few creeks are observed along the Kenya Coast i.e. Lamu creeks, Mtwapa creek, Mombasa creeks and Mida creek.

The coastal geomorphology is markedly influenced by only two permanent rivers namely the Tana and Sabaki rivers which empty into Ungwana and Malindi bays respectively. Both rivers originate from the volcanic highlands around Mt. Kenya and the Aberdares Range. There are also a few seasonal rivers which together with the permanent ones, bring

in large quantities of freshwater, mud and silt into the sea during the rainy season. This results in lowered salinity and an increase in turbidity of the water near river mouths. In the estuaries and in the smaller creeks an extensive brackish water zone is often found.

## 2.3. Hydrography

Knowledge of the types of water systems along the east coastal region is crucial in understanding the behaviour of the organisms that inhabit the area. Furthermore, the flow of water in the coastal zone is the vehicle for the fluxes of dissolved nutrients which are a necessity for the flora.

The most important water body that influences the Kenya coast is the South Equatorial Current (SEC). This current flows across the Indian Ocean from east to west and reaches the African coast near Cape Delgado in Mozambique, about 11°S latitude. It then divides into a Southward flowing stream, the Mozambique current and the northward flowing East African coastal Current (EACC) (Fig 5). The EACC forms the whole of the surface water in this region and influences the conditions in the upper layer of the seas as it flows northwards throughout the year along the coast of East Africa.

The extent to which the EACC goes northwards, its velocity, vertical thickness and salinity are all affected by the two major alternating monsoonal seasons whose belt the region lies within. During the South-East monsoon, from April to October/November, the winds are predominantly south-easterly (Figure 6).

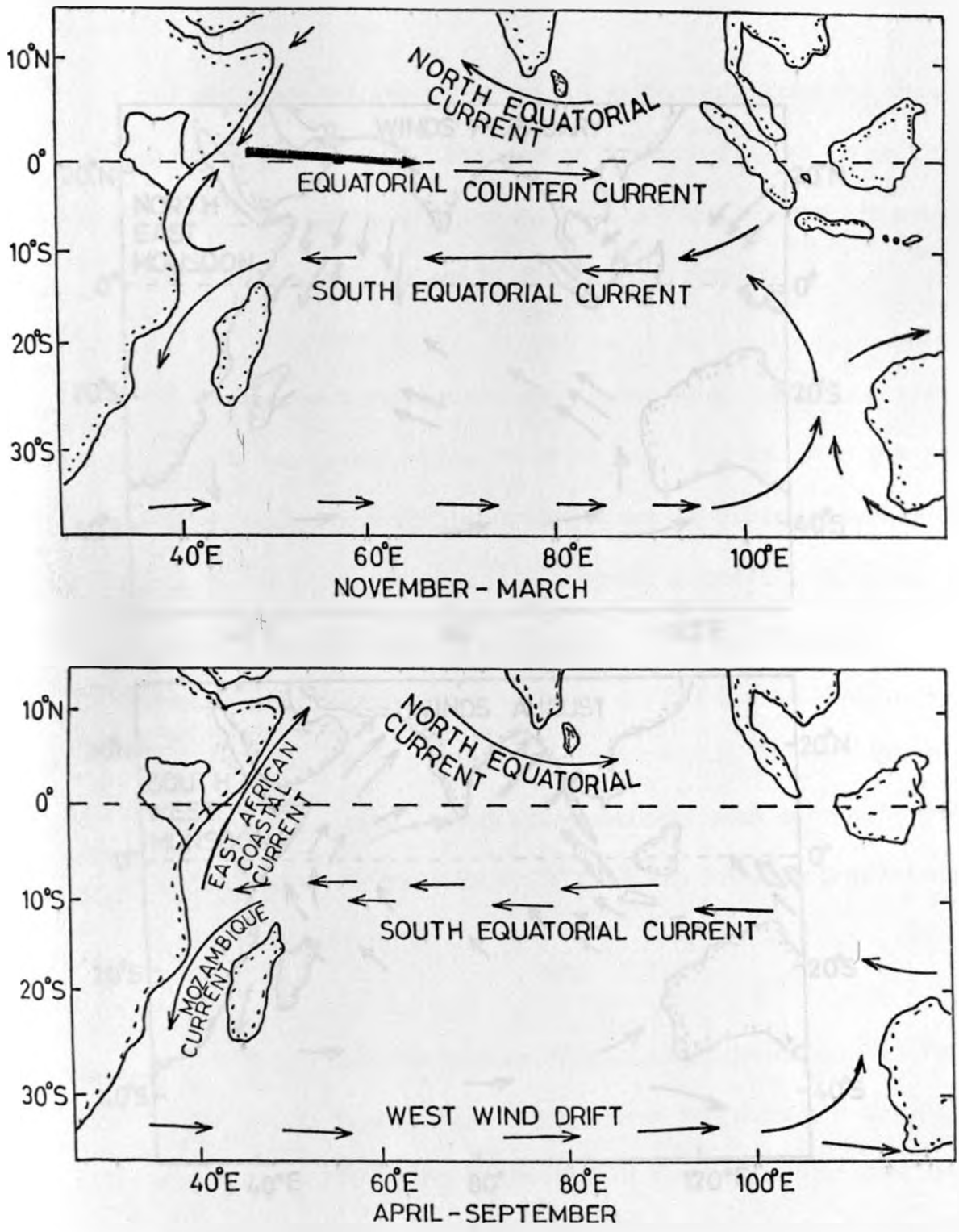



Fig. 5. The surface currents in the Indian ocean (after Newell 1957).

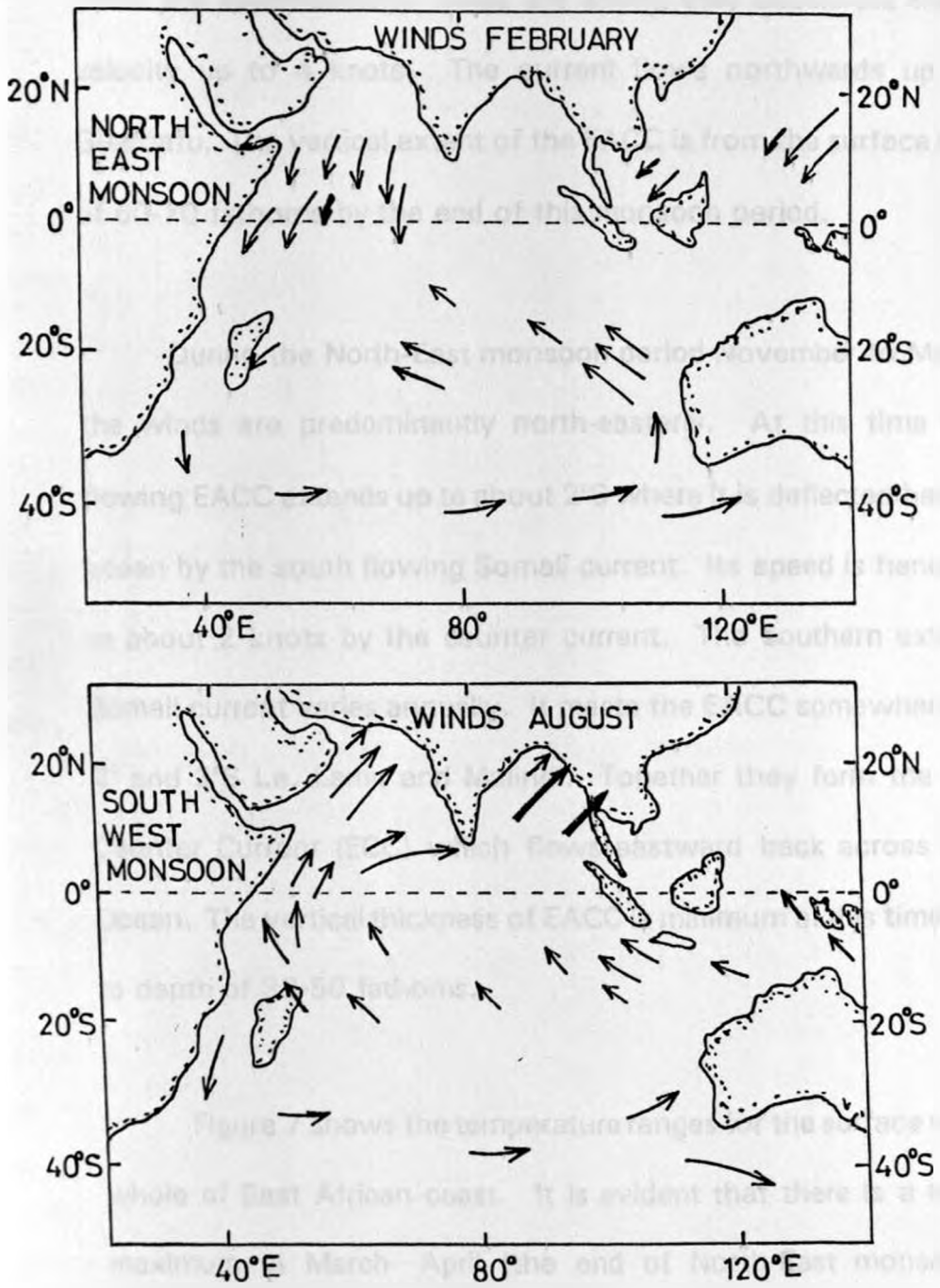



Fig. 6. The Monsoon wind distribution during February and August (After Düing 1970).

Since the south-easterly winds are strong they accelerate the current velocity up to 4 knots. The current flows northwards up to Carpe Guardafu. The vertical extent of the EACC is from the surface to a depth of 60-70 fathoms by the end of this monsoon period.

During the North-East monsoon period November to March/April, the winds are predominantly north-easterly. At this time the north flowing EACC extends up to about 2°S where it is deflected back into the ocean by the south flowing Somali current. Its speed is hence reduced to about 2 knots by the counter current. The southern extent of the Somali current varies annually. It meets the EACC somewhere between 2° and 3°S i.e. Lamu and Malindi. Together they form the Equatorial Counter Current (ECC) which flows eastward back across the Indian Ocean. The vertical thickness of EACC is minimum at this time extending to depth of 30-50 fathoms.

Figure 7 shows the temperature ranges for the surface water of the whole of East African coast. It is evident that there is a temperature maximum in March- April (the end of North-East monsoon) and a temperature minimum in August- September (end of South-East monsoon). Under the surface is a very marked thermocline both in the

north and south at all seasons. However, the depth of the thermocline varies from station to station and from season to season. During the northern monsoon the surface water is so stable that up to three thermoclines may appear (Newell, 1957).

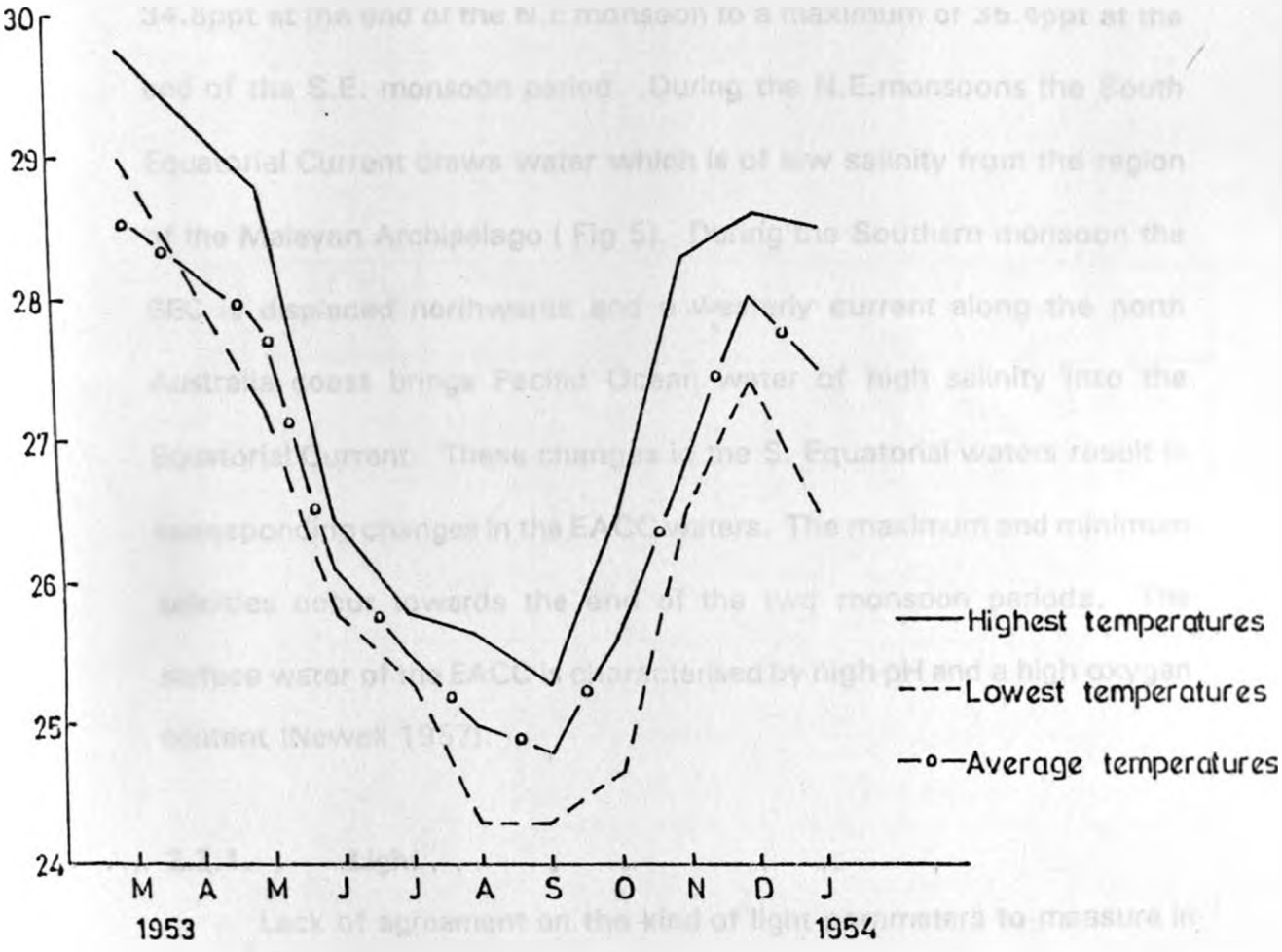



Fig. 7 . Temperature ranges of the surface waters of east coast of Africa ( After Newell 1957 )

The seasonal pattern of salinity varies from a minimum of about 34.8ppt at the end of the N.E monsoon to a maximum of 35.4ppt at the end of the S.E. monsoon period. During the N.E.monsoons the South Equatorial Current draws water which is of low salinity from the region of the Malayan Archipelago ( Fig 5). During the Southern monsoon the SEC is displaced northwards and a westerly current along the north Australia coast brings Pacific Ocean water of high salinity into the Equatorial Current. These changes in the S. Equatorial waters result in corresponding changes in the EACC waters. The maximum and minimum salinities occur towards the end of the two monsoon periods. The surface water of the EACC is characterised by high pH and a high oxygen content (Newell 1957).

### 2.3.1. Light

Lack of agreement on the kind of light parameters to measure in the sea and the means to employ in order to obtain measurements of the greatest value, has presented many difficulties on light measurements in the marine environment. However, transparency as determined by simple instruments such as secchi disk has been widely used. Transparency of the waters within the boundary zone between the equatorial current and the equatorial counter current, a region which embraces the area under study, is reported to be less than 30m (relative transparency) (McGill, 1973), Figure 8.

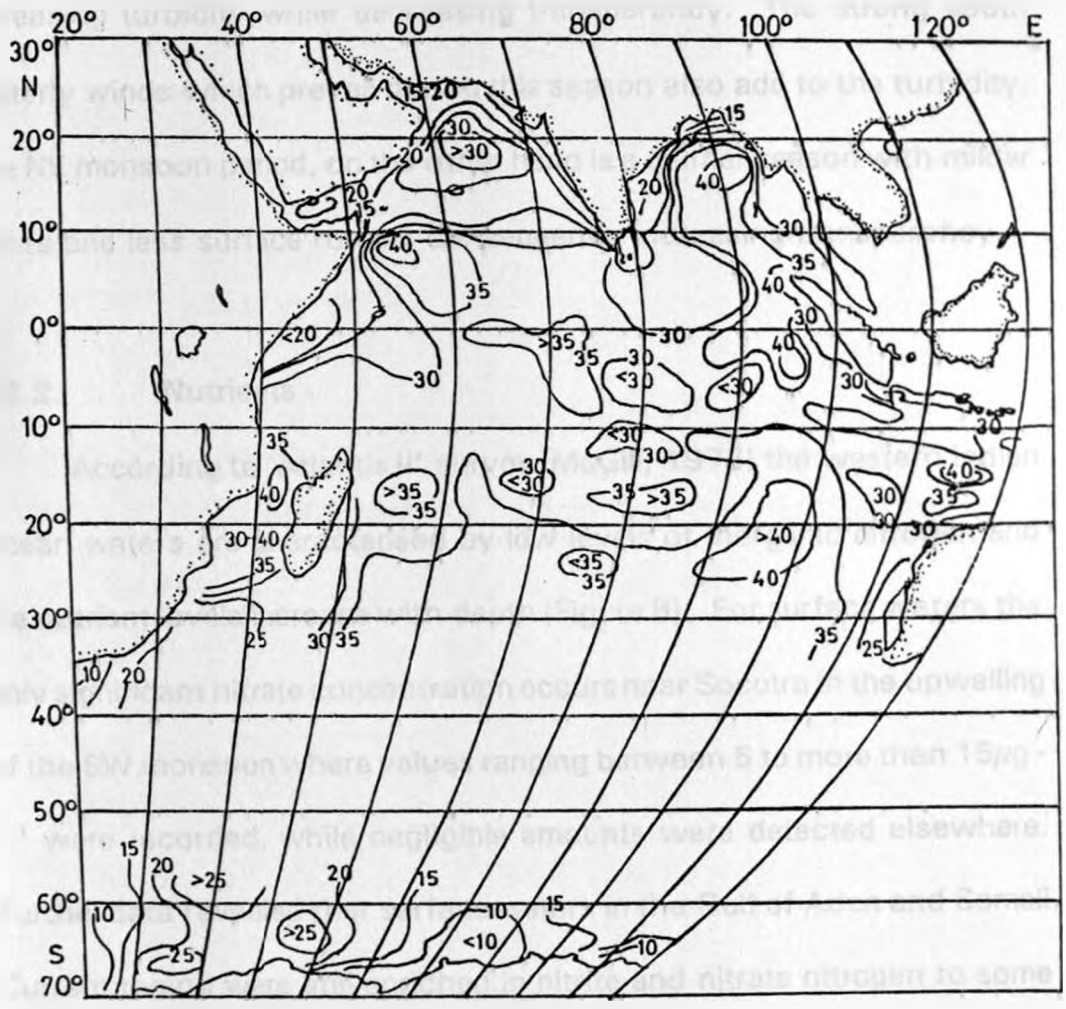



Fig. 8. Relative transparency ( m ) of Indian Ocean waters ( after Mc Gill, 1973 ).

The influence of the monsoon circulation on the movement of surface waters in the region is a major factor affecting transparency in the region. The SE monsoons bring with it the long rains which increase surface run-off and river discharges from land to the coastal waters thus increasing turbidity while decreasing transparency. The strong south easterly winds which prevail during this season also add to the turbidity. The NE monsoon period, on the other hand is a calmer season with milder winds and less surface run off consequently increasing transparency.

### 2.3.2. Nutrients

According to 'Atlantis II' survey (McGill, 1973) the western Indian Ocean waters are characterised by low levels of inorganic nitrogen and the nutrient levels increase with depth (Figure 9). For surface waters the only significant nitrate concentration occurs near Socotra in the upwelling of the SW monsoon where values ranging between 5 to more than 15$\mu g - l^{-1}$ were recorded, while negligible amounts were detected elsewhere. Further data revealed that surface waters in the Gulf of Aden and Somali Current region were still enriched in nitrite and nitrate nitrogen to some extent in the post-monsoon season (October to April).

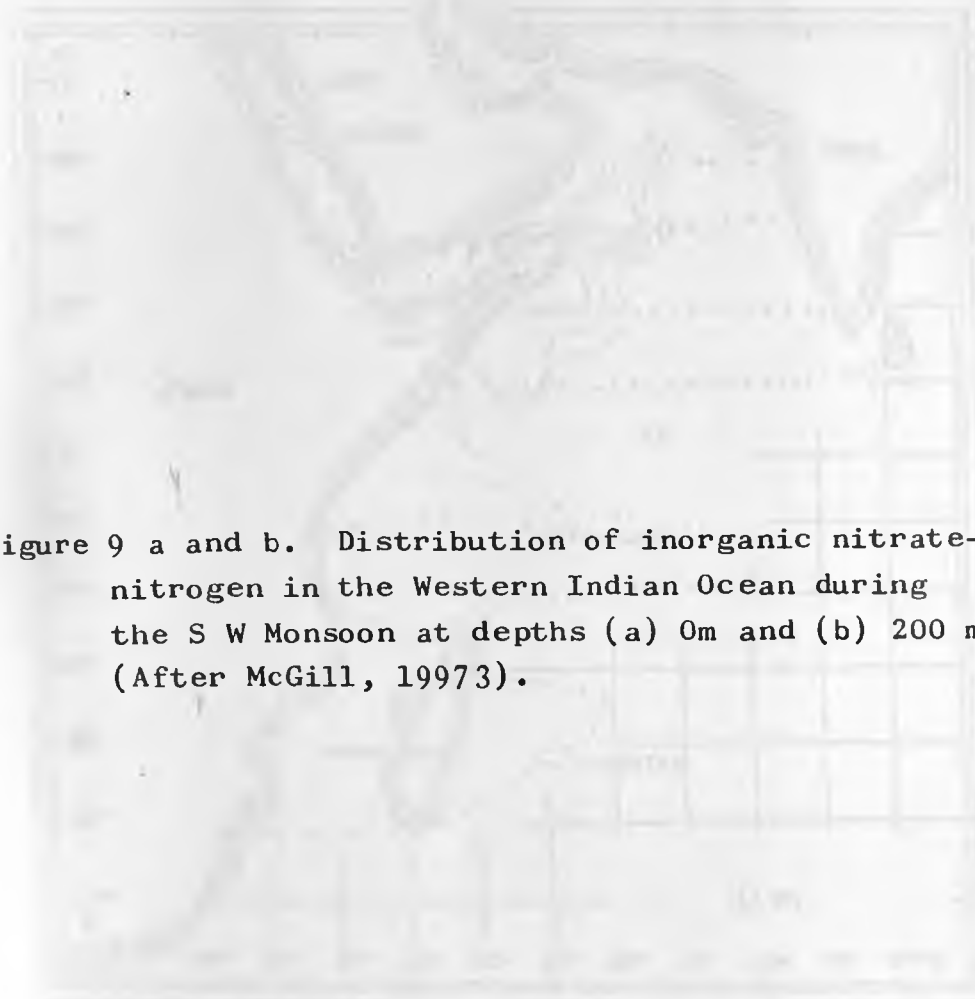


Figure 9 a and b. Distribution of inorganic nitrate-nitrogen in the Western Indian Ocean during the S W Monsoon at depths (a) 0m and (b) 200 m. (After McGill, 19973).

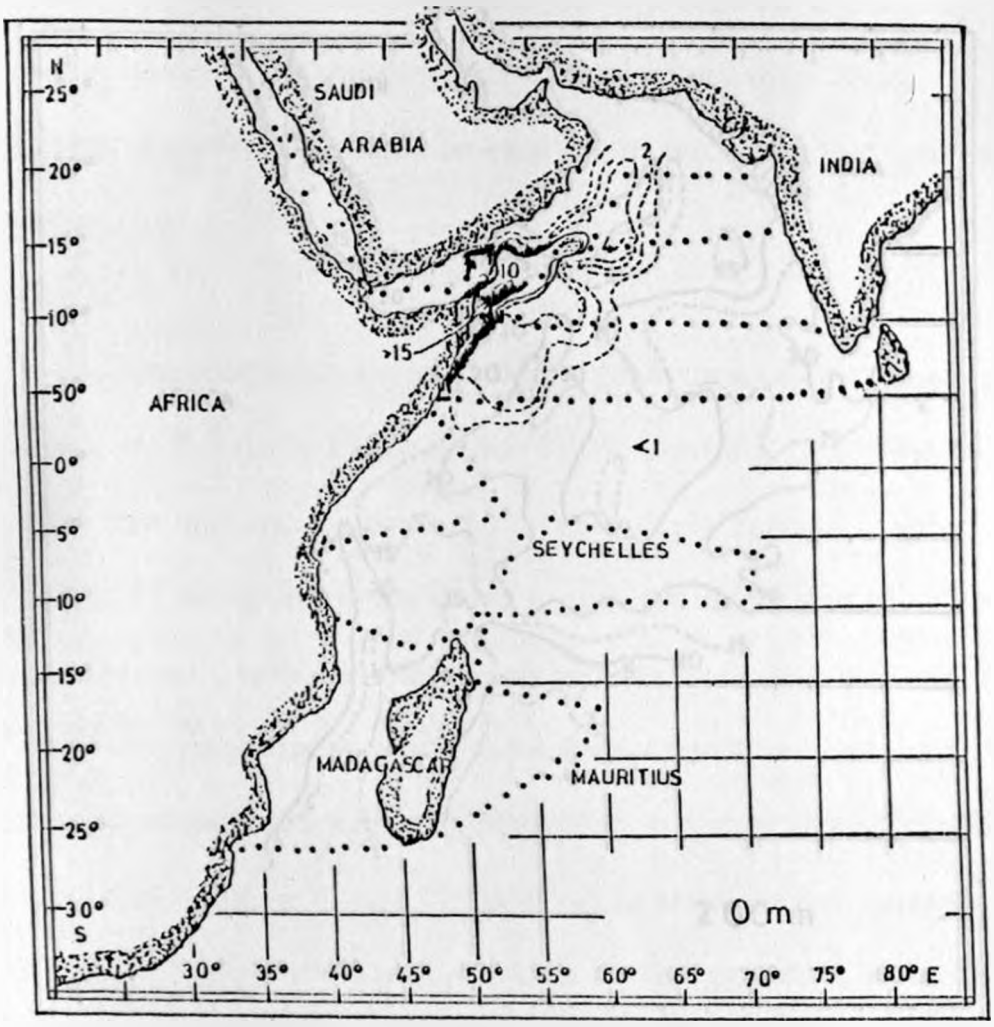

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Fig.9. (a)

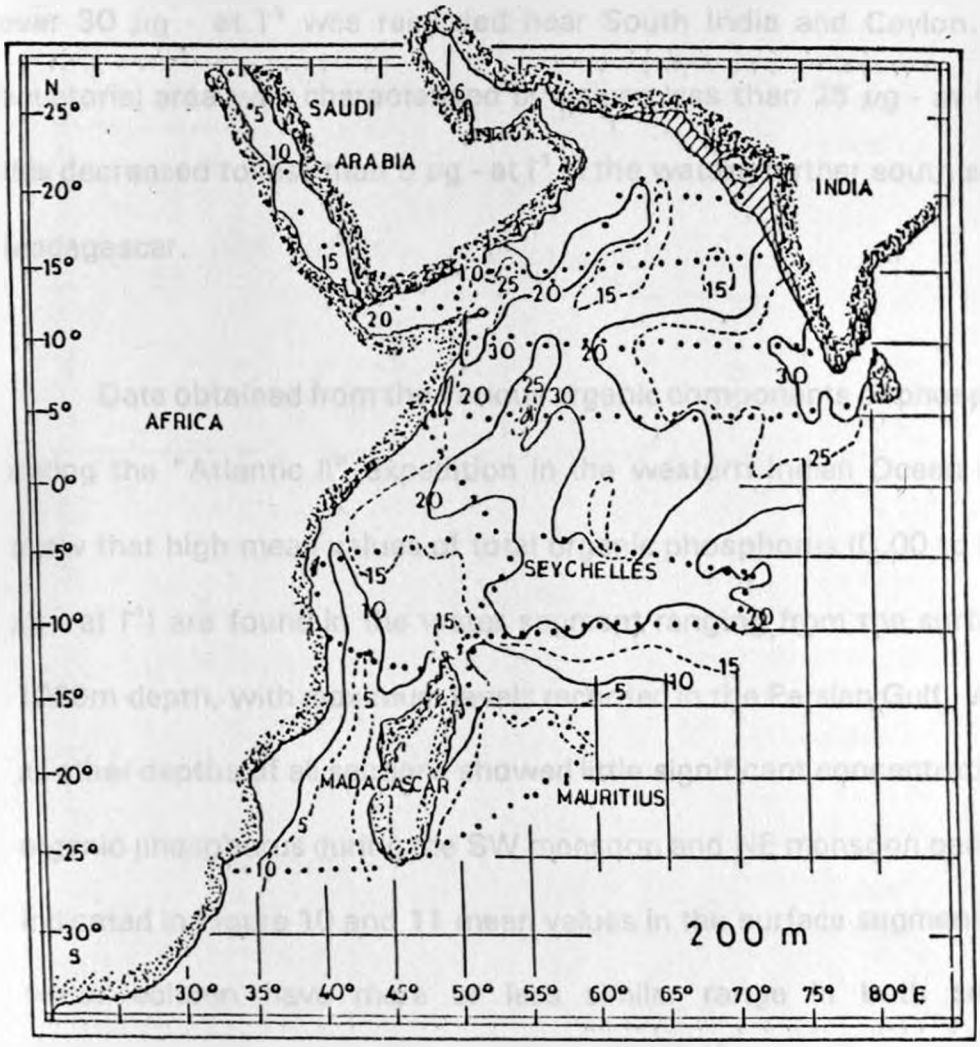
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Fig. 9 (b)

Data from the expedition showed that the western Arabian Sea had the highest nitrate levels at 200m (over 25$\mu$g - at $l^{-1}$). A maximum of over 30 $\mu$g - at $l^{-1}$ was recorded near South India and Ceylon. The equatorial area was characterised by values less than 25 $\mu$g - at $l^{-1}$ and this decreased to less than 5 $\mu$g - at $l^{-1}$ in the waters further south around Madagascar.

Data obtained from the various organic components of phosphorus during the "Atlantic II" expedition in the western Indian Ocean region show that high mean values of total organic phosphorus (0.00 to 0.185 $\mu$g - at $l^{-1}$) are found in the water segment ranging from the surface to 1000m depth, with maximum levels recorded in the Persian Gulf. Almost all other depths of all sections showed little significant concentrations of organic phosphorus during the SW monsoon and NE monsoon period are indicated in Figure 10 and 11 mean values in the surface segment of the water column have more or less similar range in both seasonal distribution, while areas of lower depths of the water column in the southern Indian Ocean showed consistently higher values than those in the Arabian sea during the NE monsoon and the case was reversed for the SW monsoon.

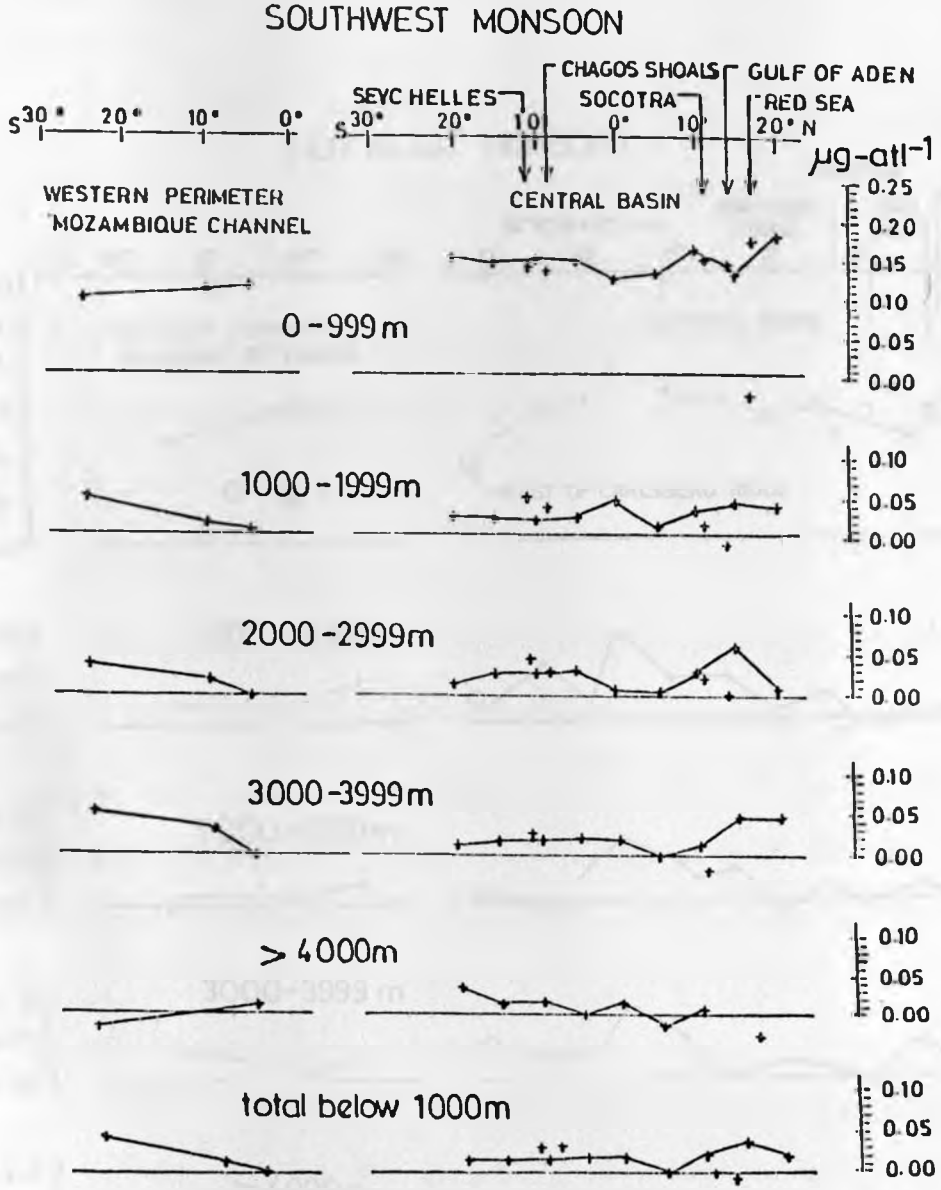



Fig.10. Distribution of total organic phosphorus in western Indian Ocean during the south west monsoon period (after Mc Gill, 1973)

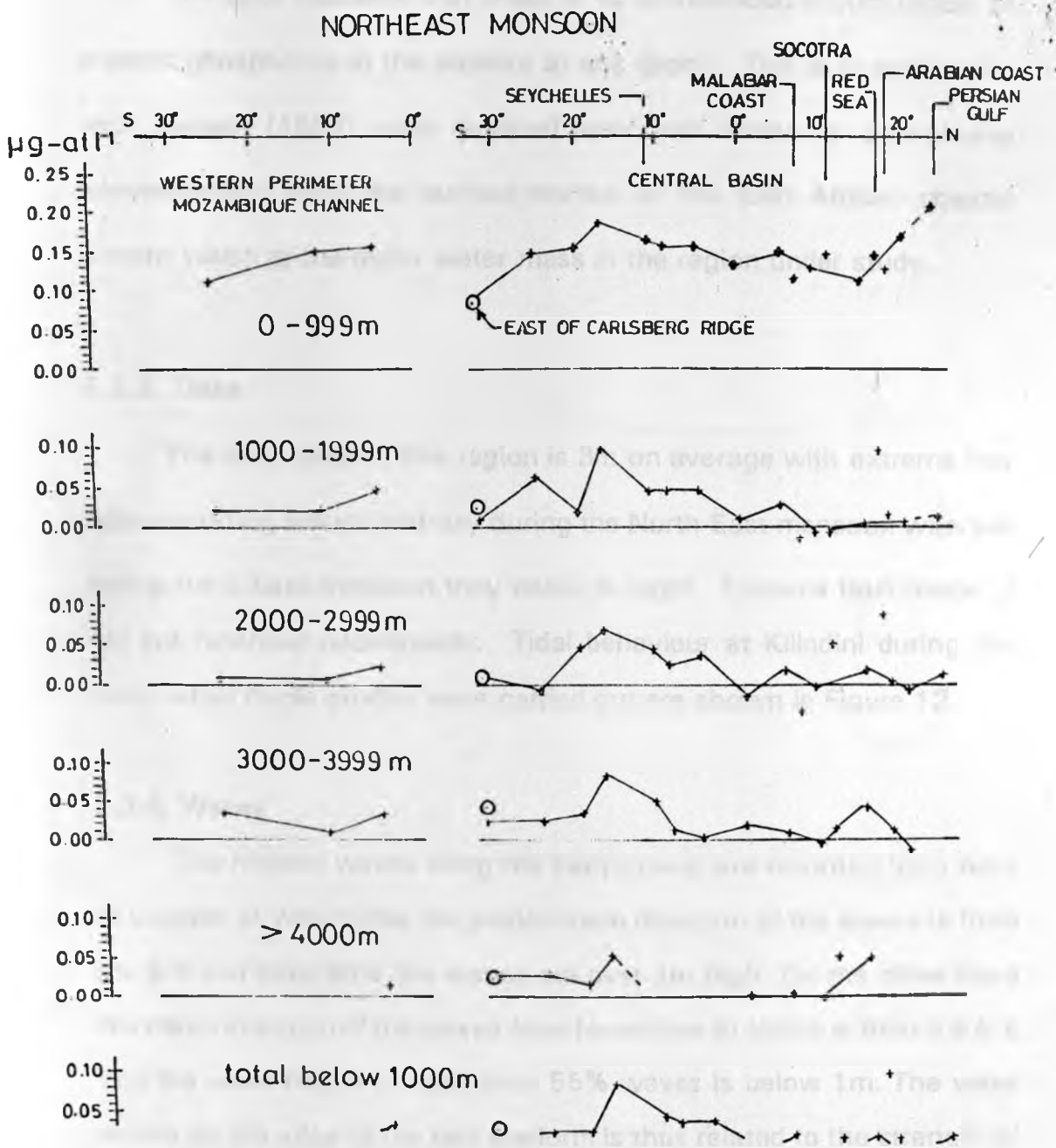



Fig.11. Distribution of total organic phosphorus in the Western Indian Ocean during the NE. monsoon period (after McGill, 1973).

The data indicates that there is no pronounced accumulation of organic phosphorus at the equator at any depth. This is in conformity with Newell (1957) who reported very low levels of phosphorus concentrations from the surface waters of the East African coastal current which is the major water mass in the region under study.

### 2.3.3. Tides

The tidal range in this region is 3m on average with extreme low tides occurring around mid-day during the North-East monsoon whereas during the S-East monsoon they occur at night. Extreme tidal range of 4m are recorded occasionally. Tidal behaviour at Kilindini during the years when these studies were carried out are shown in Figure 12.

### 2.3.4. Waves

The highest waves along the Kenya coast are recorded from April to October at which time the predominant direction of the waves is from the S-E and over 60% the waves are over 1m high. On the other hand the major direction of the waves from November to March is from the N-E and the wave height of more than 55% waves is below 1m. The wave action on the edge of the reef platform is thus related to the strength of the monsoonal winds. Coral reefs act as wave breakers where they exist along the shoreline and hence most lagoons, reef flats and intertidal pools are quite calm throughout the year.

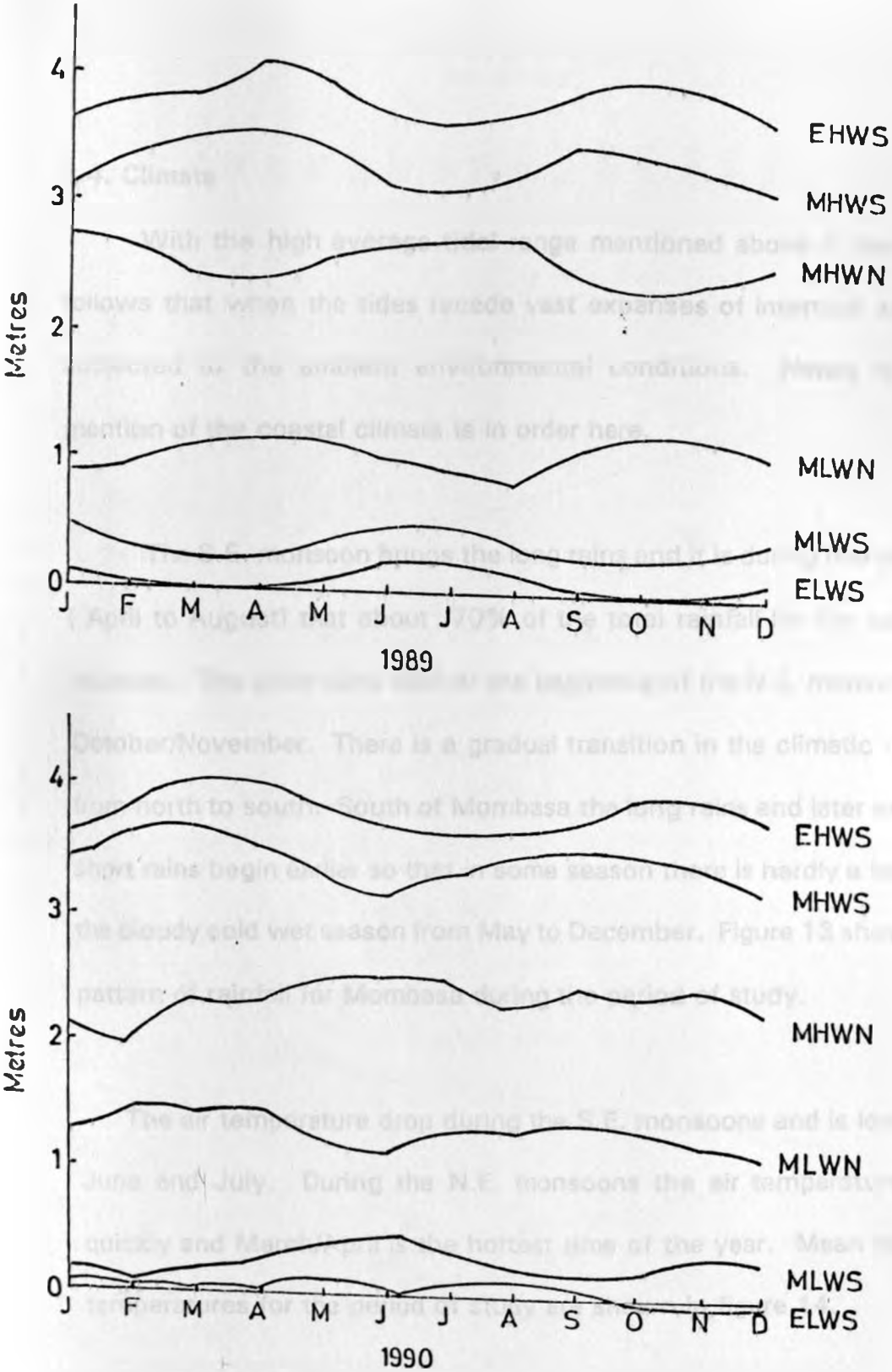



Fig.12. The behaviour of tides at Kilindini during 1989 and 1990 based on tide tables for the station

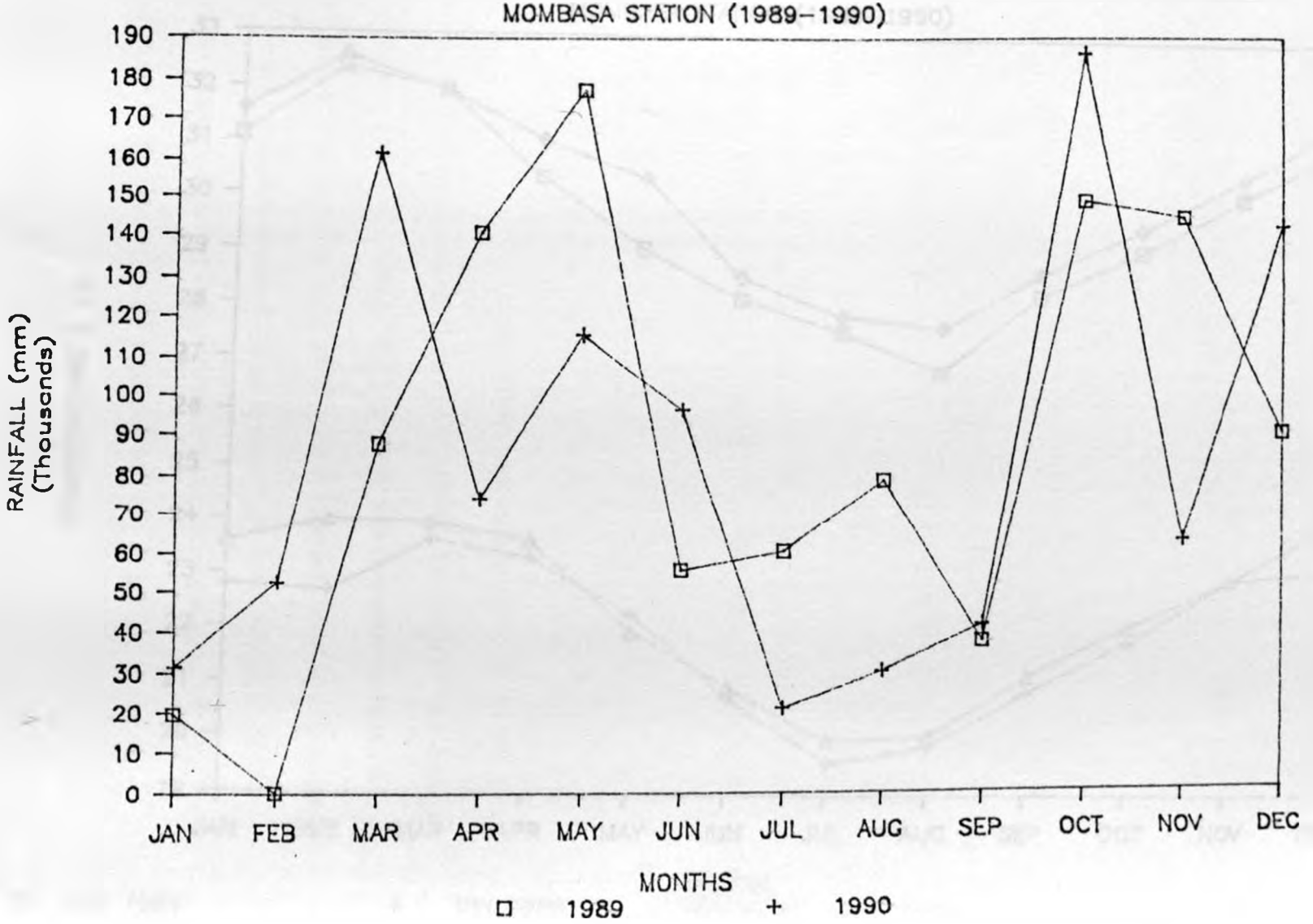
## 2.4. Climate

With the high average tidal range mentioned above it therefore follows that when the tides recede vast expanses of intertidal zone is subjected to the ambient environmental conditions. Hence a brief mention of the coastal climate is in order here.

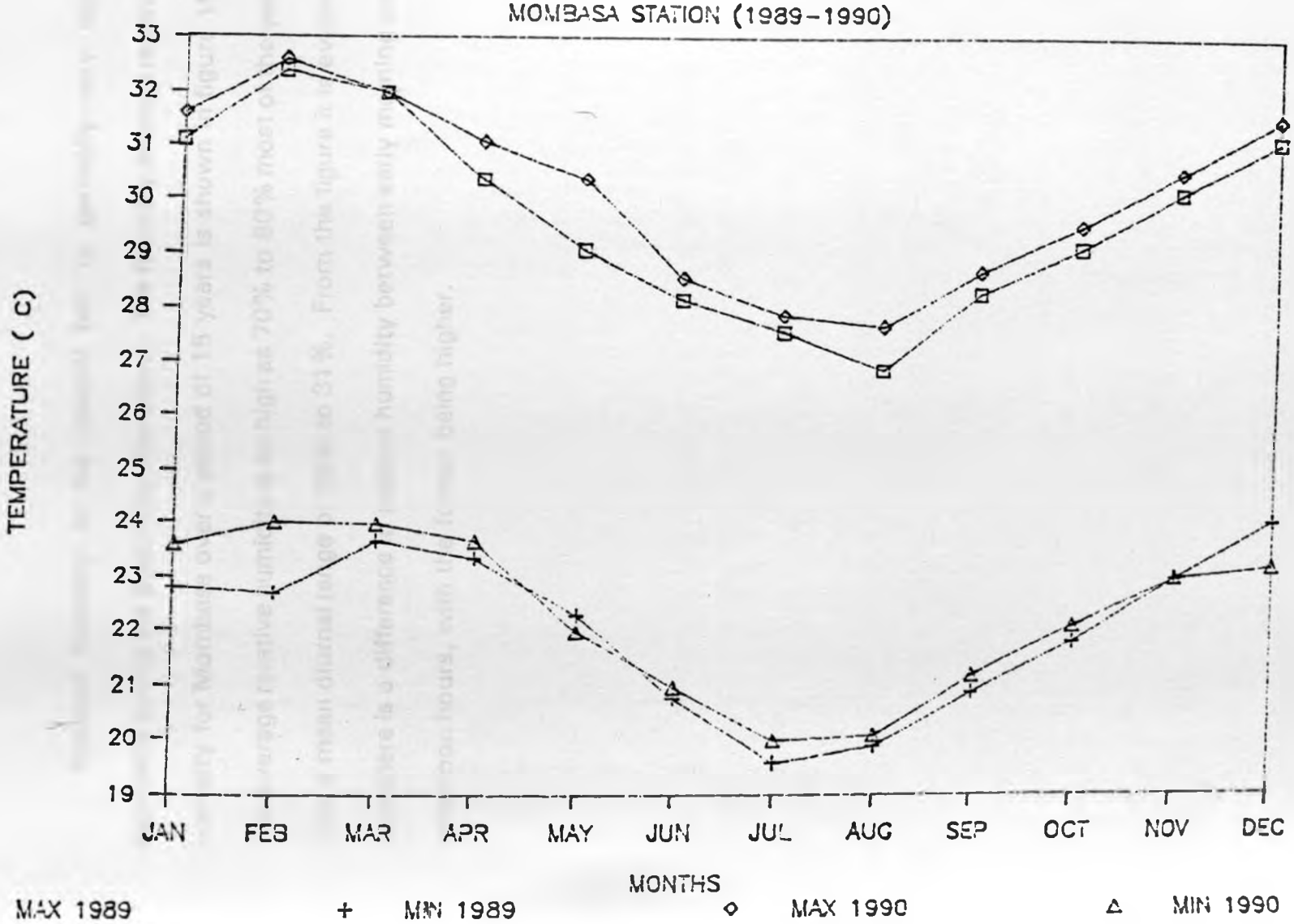
The S.E. monsoon brings the long rains and it is during this season ( April to August) that about 70% of the total rainfall for the coast is received. The short rains start at the beginning of the N.E. monsoons in October/November. There is a gradual transition in the climatic regime from north to south. South of Mombasa the long rains end later and the short rains begin earlier so that in some season there is hardly a break in the cloudy cold wet season from May to December. Figure 13 shows the pattern of rainfall for Mombasa during the period of study.

The air temperature drop during the S.E. monsoons and is lowest in June and July. During the N.E. monsoons the air temperature rises quickly and March/April is the hottest time of the year. Mean monthly temperatures for the period of study are shown in figure 14.

# FIG. 13: TOTAL MONTHLY RAINFALL



# FIG. 14: MEAN MONTHLY AIR TEMPERATURES



Relative humidity at the coastal belt is generally very high especially during the South East monsoons. The monthly average relative humidity for Mombasa over a period of 15 years is shown in figure 15. The average relative humidity is as high as 70% to 80% most of the year with a mean diurnal range of 26% to 31%. From the figure it is evident that there is a difference in relative humidity between early morning and afternoon hours, with the former being higher.

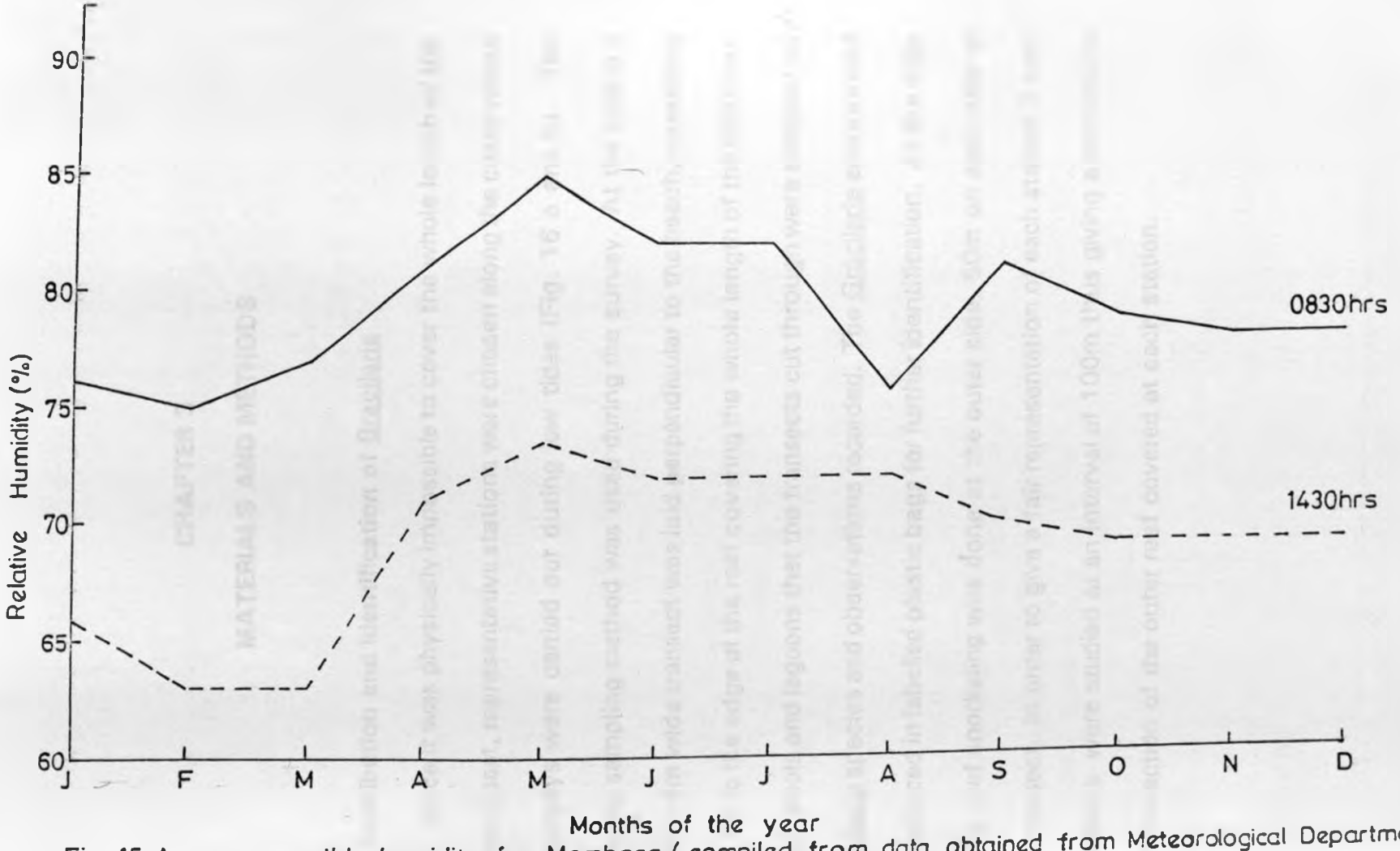



Fig .15. Average monthly humidity for Mombasa ( compiled from data obtained from Meteorological Department, Kenya )

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1. Distribution and Identification of Gracilaria

Since it was physically impossible to cover the whole length of the Kenyan coast, representative stations were chosen along the coast where the surveys were carried out during low tides (Fig. 16 a and b). The following sampling method was used during the survey. At the beach a straight 4m wide transect was laid perpendicular to the beach, stretching right up to the edge of the reef covering the whole length of the platform. All the pools and lagoons that the transects cut through were sampled for Gracilaria species and observations recorded. The Gracilaria encountered were placed in labelled plastic bags for further identification. At the edge of the reef snorkeling was done at the outer side, 50m on each side of the transect. In order to give a fair representation of each station 3 such transects were studied at an interval of 100m thus giving a continuous 300m section of the outer reef covered at each station.


Fig. 16a. The Kenya Coast showing stations surveyed. (Adapted from Oyieke and Ruwa 1987).

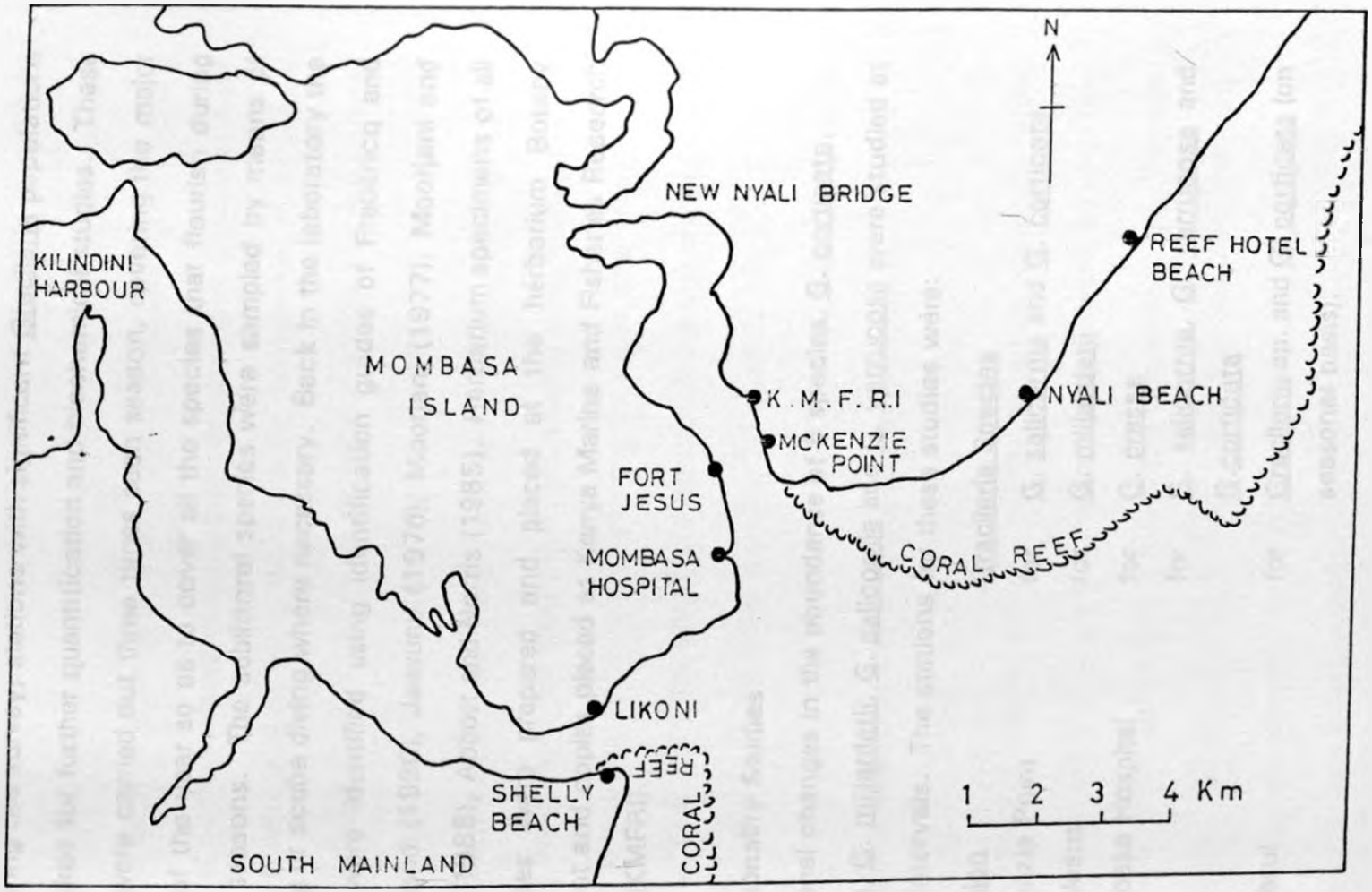



Fig. 16b. Study sites in Mombasa Area. (Adapted from Oyieke & Ruwa 1987).

During the survey, stations with significant Gracilaria populations were marked for further quantification and biochemical studies. These surveys were carried out three times each season, covering the major seasons of the year so as to cover all the species that flourish during different seasons. The sublittoral species were sampled by means of snorkeling or scuba diving where necessary. Back in the laboratory the samples were identified using identification guides of Fredericq and Hommersand (1990), Jaasund (1976), Moorjani (1977), Moorjani and Simpson (1988), Abbott and Norris (1985). Herbarium specimens of all the species were prepared and placed at the herbarium Botany department and copies placed at Kenya Marine and Fisheries Research Institute (KMFRI).

### 3.2. Seasonality Studies

Seasonal changes in the abundance of 6 species, G. corticata, G. crassa, G. millardetii, G. salicornia and G. verrucosa were studied at monthly intervals. The stations for these studies were:

| Station | | Gracilaria Species |
|---|---|---|
| McKenzie Point | for | G. salicornia and G. corticata |
| Fort Jesus | for | G. millardetii |
| Mombasa Hospital | for | G. crassa |
| Gazi | for | G. salicornia, G. verrucosa and G.corticata |
| Mambrui | for | Gracilaria sp. and G. corticata (on seasonal basis). |

To gather precise information on the changes in the quantity of algal growth, data was collected with the permanent quadrant method. 3 quadrants each with an area of 50 x 50cm were marked with chisel and hammer on the surfaces of some selected accessible boulders where they were available or by using white marine paint on the rocks where they were exposed at low tide or by permanent stakes in sandy areas. The quadrants were selected during peak periods for each species. The abundance of the algal growth was measured at 1 month interval as the percentage basal cover per quadrat. These studies were carried out for the above 6 species which were growing in areas that were easily accessible. For G. corticata since the thalli were huge and covering large areas in comparison to the other species, the quadrat size was 1 x 1m. At each data collection time each quadrat was divided into 4 so as to facilitate percentage basal cover estimation by inspection.

The heights above datum, of the beaches sampled, were determined from observations made during calm waters around neap tide days, using tidal predictions for 1990 produced by Physical Oceanography Section of KMFRI. At these stations, the following parameters were measured each time sampling was done:

a Salinity, using a refractometer

b Water temperature, using ordinary thermometer

c Water samples fixed using mercuric chloride and transported back to the laboratory in a portable freezer, for nitrate and phosphate content analysis. The samples were stored at - 8°C.

These parameters were measured both from marked intertidal pools and Ocean water.

### 3.3. Growth Studies

A study was carried out to find out the rate of growth of G. corticata and how long it takes to grow to maturity. The study commenced when new thalli germination was observed. 30 thalli were collected on weekly intervals placed in plastic bags and taken back to the laboratory where each thallus was blotted dry placed in a separate petri dish and its wet weight recorded. These were then placed in an oven at 50°C and dried to a constant weight which was also recorded. The study was carried out over the entire life span of the population.

### 3.4. Nitrate Analysis

The methods adapted for nitrate determination are those of Parsons et. al. (1984) and Eppley (1978). The principle behind the

measurement of nitrates in this study is based on the method of reducing nitrate to nitrite by coarse cadmium metal filings plated with copper.

### 3.4.1. Reagent Preparation

**Reduction Column.**

About 100g of cadmium - copper filings was stirred in 500ml of 2% w/v solution of copper sulphate ($CuSo_4$. $5H_2O$) until the blue colour left the solution. A small plug of copper wool was placed in the bottom of the reduction column and the column filled with dilute $NH_4Cl_2$ solution. The slurry of the cadmium copper filling was then poured in and the column gently packed to a height or about 30cm. It was ensured that the filings are permanently covered with dilute $NH_4Cl_2$. The column was then washed thoroughly with dil. $NH_4Cl_2$ and the flow rate adjusted by tapping the side of the column so that about 100ml was collected in 8-12 minutes. Figure 17.

**Sulfanilamide Solution**

5g of sulfanilamide was dissolved in a mixture of 50ml conc. HCl and about 300ml distilled water. This was further diluted to 500 ml with distilled water.

**C-(1-Naphthyl)-Ethylinediamine Dihydrochloride Solution**

0.5g of the dihydrochloride was dissolved in 500 ml distilled water. The solution was stored in a dark bottle and renewed once a month.

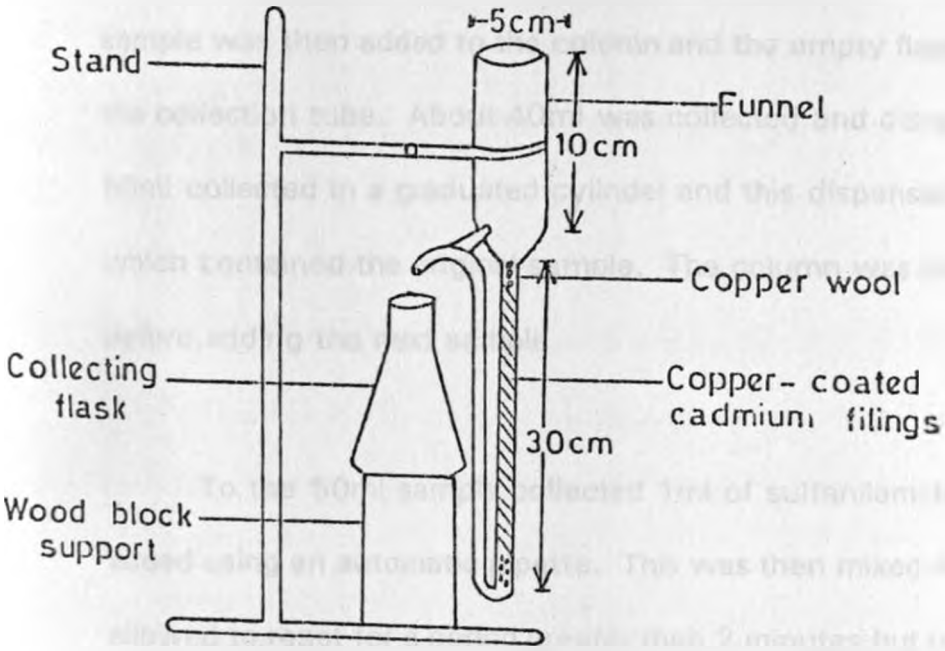



Fig 17. Nitrate reduction column showing dimensions.

### 3.4.2. Experimental Procedure

2ml of Conc. $NH_4Cl_2$ was added to 100ml of the sea water sample in an Erlenmeyer flask. The solution was mixed and about 5ml poured onto the top of the column and allowed to pass through. The remaining sample was then added to the column and the empty flask placed under the collection tube. About 40ml was collected and discarded and later 50ml collected in a graduated cylinder and this dispensed into the flask which contained the original sample. The column was allowed to drain before adding the next sample.

To the 50ml sample collected 1ml of sulfanilamide solution was added using an automatic pipette. This was then mixed and the reagent allowed to react for a period greater than 2 minutes but not exceeding 8 minutes. Then 1ml of naphthylenediamine solution was added and mixed immediately. The extinction of the solution was then measured between 10 minutes and 2 hours afterwards in a 1cm cuvette against distilled water using a wavelength of 543nm on a UV-150-02 spectrophotometer.

The observed extinction was corrected by that of the reagent blank, see 3.4.3 below, and the concentration of nitrate calculated by using regression.

### 3.4.3. Determination of Blank

The procedure in 3.4.2 was carried out using 100ml of dilute $NH_4Cl_2$ instead of the seawater sample. The extinction was then measured using the same cuvette as used for the seawater samples. Extinction of three blanks were taken and an average determined. This average value was subtracted from the extinctions of the samples.

### 3.4.4. Calibration

Synthetic seawater was prepared by dissolving 310g of analytical reagent quality NaCl, 100g of reagent quality $MgSO_4$ $7H_2O$ and 0.5g of $NaHCO_3$. $H_2O$ in 10 litres of distilled water.

Standard nitrate solution was prepared by dissolving 1.02g of analytical reagent $KNO_3$ in 1000ml distilled water. 4ml of this solution was diluted to 2000ml with synthetic seawater. This solution was stored in a dark bottle and prepared fresh each time it was needed.

100ml of the dilute standard nitrate solution was placed in a 125ml Erlenmeyer flask and the procedure in 3.4.2 carried out. The extinction for each individual column was noted.

### 3.5. Determination of Phosphates

The procedure followed for the determination of phosphates in the water samples is that of Parsons et al. (1984).

#### 3.5.1. Reagent Preparation

**Ammonium molybdate solution**

15g of analytical reagent grade ammonium paramolybdate [$(NH_4)_6M_{07}.4H_2O$] was dissolved in 500ml of distilled water. This was stored in a plastic bottle away from direct sunlight.

**Sulphuric acid solution**

140ml of concentrated analytical reagent quality sulfuric acid was added to 900ml of distilled water. The solution was then allowed to cool then stored in a glass bottle.

**Ascorbic acid solution**

27g of ascorbic acid was dissolved in 500ml of distilled water. This was stored in a plastic bottle and frozen solid in the freezer.

**Potassium antimonyl - tartrate solution**

0.34g of potassium antimonyl tartrate (tartar emetic) was dissolved in 250ml of distilled water with slight warming. This was then stored in

a glass bottle.

**Mixed reagent**

100ml ammonium molybdate, 250ml sulfuric acid, 100ml ascorbic acid and 50ml of potassium antimonyl tartrate solutions were mixed together. This reagent was only prepared when needed.

### 3.5.2. Experimental Procedure

Previously frozen water sample was allowed to thaw at room temperature. Using a syringe-type pipette 10ml of the mixed reagent was added to 100ml of the thawed water sample and mixed at once. After 5 minutes and within the first 2-3 hours the extinction was measured in a 10cm cell against distilled water at 885nm on a UV-150-20 spectrophotometer. The extinction was corrected with the reagent blank (and turbidity blank where necessary) and phosphate concentration extrapolated using the calibration curve determined as shown below.

### 3.5.3. Determination of Blank

Distilled water was used in place of sample and experimental procedure carried out as above to obtain the extinction of the reagent blank.

### 3.5.4. Calibration

A stock solution was prepared by dissolving 0.136g of anhydrous potassium dihydrogen phosphate ($KH_2PO_4$) in 1 liter of distilled water. The concentration of this solution was 1000$\mu$g at P/l. The stock solution was stored in a dark bottle with 1ml of chloroform. From this stock solution dilution series were prepared so as to give the following concentrations: 0.1; 0.5; 1.0; 1.5; 2.0; $\mu$g at. P/l. These solutions were taken through the experimental procedure as in 3.5.2 above. The extinctions were read off and a phosphate calibration curve drawn from these. A fresh calibration curve was determined for each day of phosphate determination.

### 3.6. Agar Extraction.

As mentioned in the literature review it is known that the gel properties of many Gracilaria agars can be improved by treatment with alkali. However, in the present study, the native, unmodified agars were studied for two reasons. Firstly if a strongly gelling agar can be obtained without an alkali treatment step, it may be more attractive from an economic standpoint. Secondly, to learn more about the natural state of the polysaccharides in the algae it was desirable to first investigate the native agars. However, for the purposes of comparison agar was also

extracted from alkali treated plants and compared with the native agar, while native agar was alkali treated and properties tested too.

The method used for the extraction of the native agar was as follows: Samples were collected and sorted out, cleaned in the seawater and dried partly in the sun and partly in the oven at 50°C to a constant weight. They were then stored in a dry place. During the extraction the previously dried algae was thoroughly and quickly washed in tap water and dried partly in the sun and partly in the oven at 50°C to a constant weight. The dried algae were then ground into smaller pieces using pestle and mortar. 20g of the powder was then hydrated in 500ml distilled water overnight. This was then boiled in a water bath for 3 hours at a temperature of 95°C, with continuous stirring and replenishing evaporating water, making a final volume of 700ml. Using a double layer of cheese cloth the hot extract was filtered into a 1 liter beaker. The residue was re-extracted with 100ml of hot distilled water and filtered into the original beaker. While still hot the extract was filtered through suction pressure using analytical filter paper. Care was taken to ensure that the filtering system was kept warm in order to avoid gelling. The extract was then poured into plastic trays where it was left to gel at room temperature.

The gel was then kept in a freezer at -8°C for 24 hours. This was then removed, thawed at room temperature and the extract dissolved in 300ml of hot distilled water. This was kept at room temperature to cool into a gel which was again placed in the freezer for 24 hours. The thawing and freezing was repeated once more and the final gel dried in the oven at 50°C to a constant weight. The weight of the dried agar extract was noted then broken into small flakes and stored in dry petri dishes for further agar analysis. The yield of the agar, as percentage of the dry weight, was determined as follows:

$$\% \text{ yield} = \frac{\text{weight of dry agar}}{\text{weight of dry algae}} \times 100$$

**3.6.1. Alkali Treatment of Plants.**

20g of the dried whole algae was placed in a beaker containing 700ml of an aqueous solution of 2% NaOH and heated at a temperature of 90°C for 3 hours. It was then completely washed with freshwater, chopped into smaller pieces and placed in a beaker containing 700ml of distilled water. The acidity was adjusted to pH 6.5 and boiled at 95°C for another 3 hours. The procedure described in 3.6 above was then followed.

### 3.6.2. Alkali treatment of native agar

The alkali treatment method adapted in this study is according to Lahaye et al. (1986). 500mg of native agar was dissolved in 100ml of distilled water by placing the flask in a water bath at 80°C for about 1hr. Then 50mg of Sodium borohydride $Na(BH_4)$ was added to the polysaccharide and the solution stirred at 80°C for 10-15min. 50ml of sodium hydroxide (NaOH) was then added to the mixture and then placed in a shaker water bath at 80°C for 3hr. The solution was then neutralised carefully with 3M HCl, in an ice bath, to pH 7 after which the sample was dialysed in a spectrapor membrane tubing (M.w. cut off, 12-14,000) against running distilled water for 48hr. The sample was then freeze-dried.

### 3.7. Agar Analysis.

### 3.7.1. Preparation of 1.5% agar solution

The warm bath shaker was started and the temperature set at 95°C. In a microwave oven 60ml distilled water was warmed in a 100ml erlenmeyer flask close to boiling temperature. The flask was covered with aluminium foil in order to reduce evaporation. 0.9g of the extract was then added to the warmed water and the flask covered with aluminium foil once again. This was then placed in the preheated water

bath for about 1hr ensuring that all the extract was dissolved. Whenever necessary the agar mixture was stirred with a rod in order to mix the extract and water completely.

### 3.7.2. Determination of Gelling Temperature

15ml of 1.5% agar solution was poured into plastic vials (4.5cm diameter) and placed directly in a beaker containing water at room temperature. Measurements were taken using the apparatus designed by O. Sandegren, Innovest AB Company, Rönnäng, Sweden (Plates 1 and 2) which automatically records the temperature of the solution as it cools and finally locks up when the gelling temperature is attained. The values were compared by those of Difco Bacto Agar prepared in the same manner and measurement taken by the same apparatus.

### 3.7.3. Determination of Melting Temperature.

10ml of 1.5% hot agar solution was placed in a 16 by 120cm test tube then a glass rod, the same diameter as a thermometer, placed in an off-center location. The gel was allowed to equilibrate at room temperature overnight then the rod replaced by the thermometer and several glass beads placed on the gel surface. The test tube was clamped in a water bath heated at 1°C per minute and the melting

temperature was recorded when the beads sunk into the bottom of the test tube.

### 3.7.4. Gel Strength Determination.

4ml of 1.5% hot agar solution was allowed to gel at room temperature in plastic vials (3.5cm diameter), enclosed in plastic jars containing humid cloth. The gels were left for a minimum of 2hr to acquire room temperature. The gel strength measurements were taken using the apparatus designed by O. Sandegren, Innovest AB Company, Rönnäng, Sweden (Plate 3 and 4), which automatically increases the load on a $1cm^2$ gel surface until the point when the gel breaks. The gel strength (GS) values were compared to those of Difco Bacto Agar prepared in the same manner.

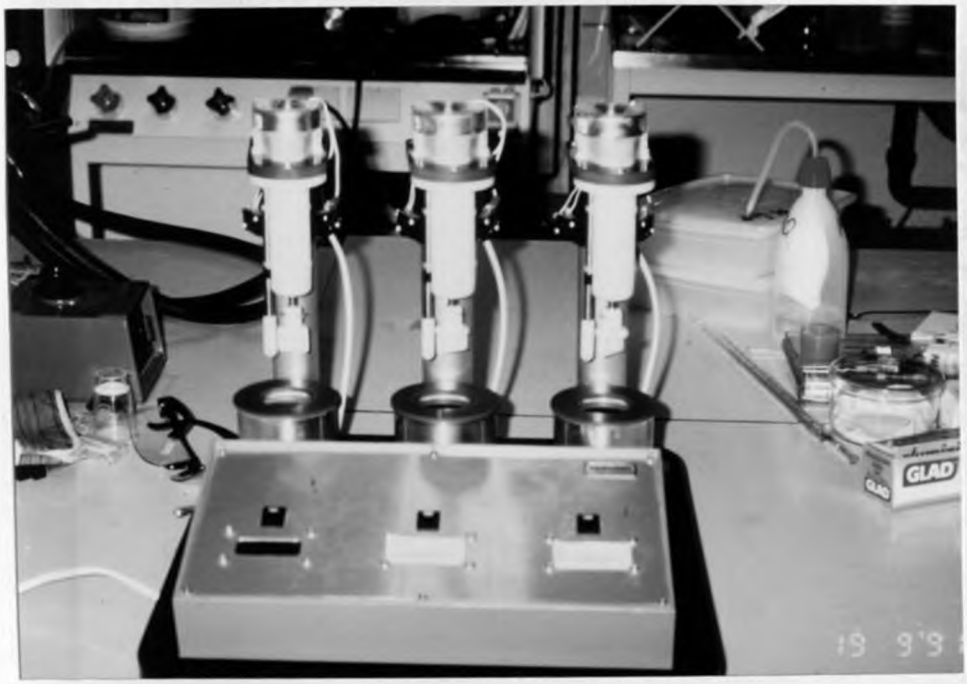



Plate 1. Top view of the apparatus used for measuring gelling temperature.

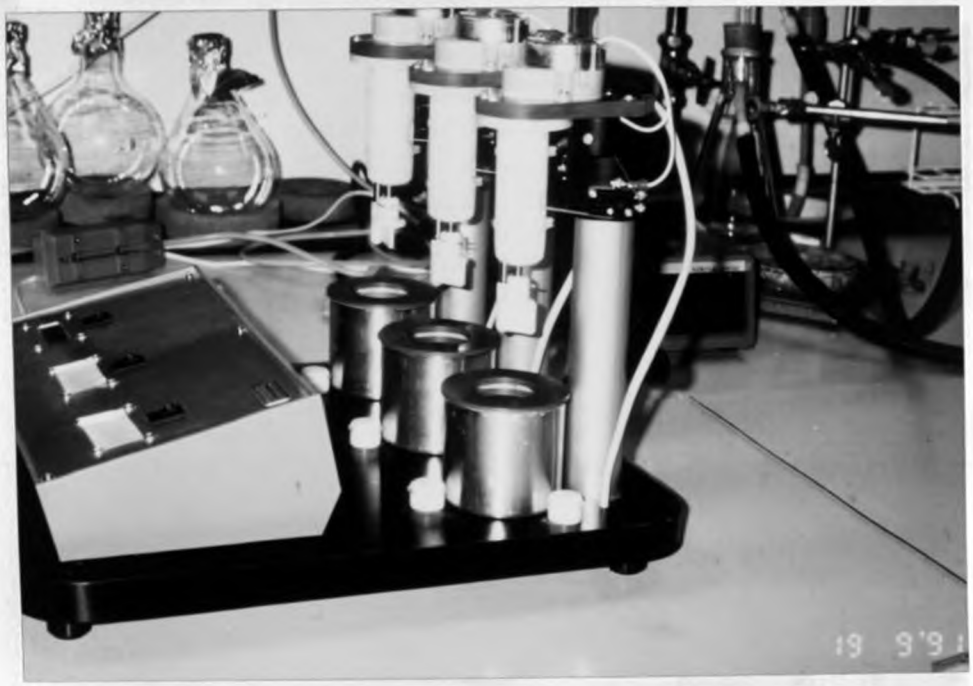


Plate 2. Side view of the apparatus used for measuring gelling temperature.

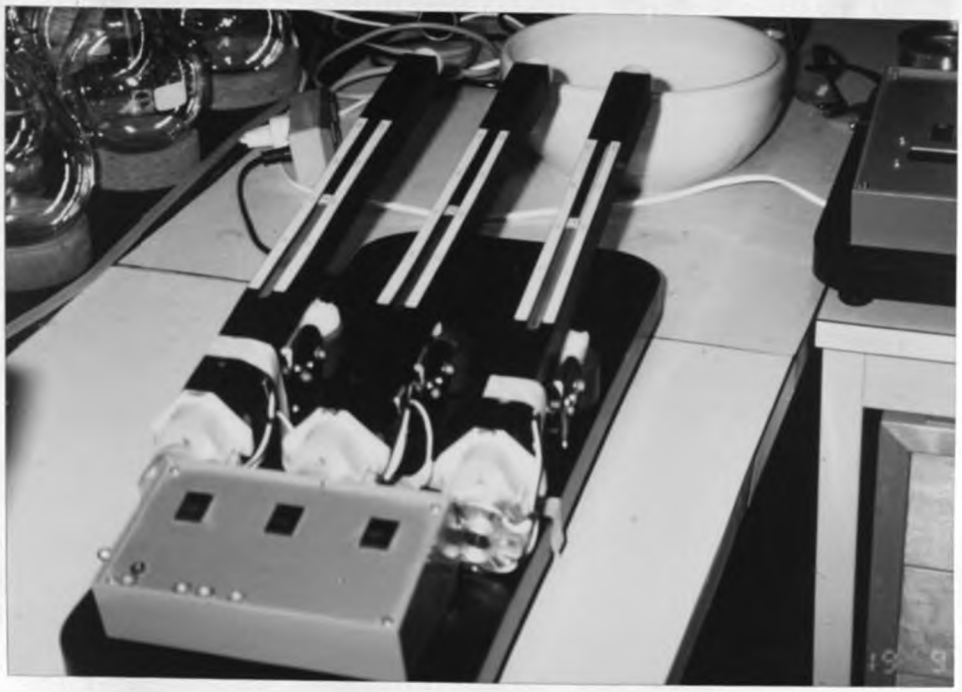


Plate 3. Top view of the apparatus used for measuring gel strength.

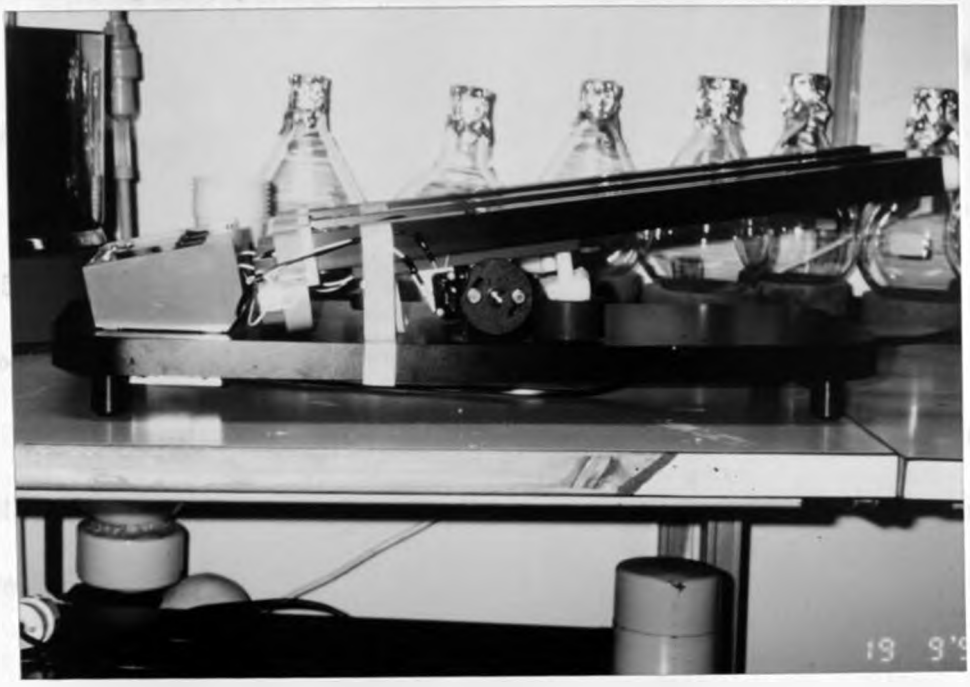


Plate 4. Side view of the apparatus used for measuring gel strength.

### 3.7.5. Determination of 3,6-Anhydrogalactose

The resorcinol reagent method of Yaphe and Arsenault (1965) was used.

#### 3.7.5.1. Sample Preparation

10mg of the sample was dissolved in 100ml distilled water by heating in a water bath at 95°C to give a concentration of 0.1mg/ml.

#### 3.7.5.2. Preparation of Reagents

**Resorcinol Stock Solution**

This was prepared by dissolving 150mg resorcinol in 100ml distilled water. It was stored in a dark bottle in a refrigerator, for not more than 1 week.

**Acetal Stock Solution**

247.5mg of acetaldehyddiethylacetal was made up to 25ml by distilled water and stored in a dark bottle in a refrigerator, for not more than 1 week. For calorimetric reagent 1ml of the stock solution was diluted to 25ml in distilled water prior to use.

**D-Fructose Stock Solution**

50mg of the sugar was dissolved in 100ml distilled water to give a concentration of 0.50mg/ml and stored in a refrigerator.

**Working Solution**

Resorcinol-acetal reagent was prepared by adding 100ml of conc. HCl to 9.0ml of the resorcinol stock solution after which 1.0ml of the diluted acetal stock solution was added. This solution was prepared fresh daily.

**3.7.5.3. Experimental Procedure**

2.0ml of each sample was placed in a 16 by 20mm test tube with a loosely fitting cap and cooled in an ice bath. Then 10ml of the cold resorcinol-acetal reagent added to each tube. This was thoroughly mixed with a tul-buzzer and warmed at 20°C for 4min then heated at 80°C for 10min. The tubes were then cooled in an ice bath for 1.5min and the absorbance read off at 555nm within 15min from a U-2000 spectrophotometer. While the colour was being developed, and thereafter, the tubes were covered by aluminium foil in order to avoid strong light.

The quantity of 3,6-anhydrogalactose was determined by reference to the standard curve for fructose (3.7.5.4 below) and multiplying this by factor 1.087. Since the concentration of the sample solution was known the percentage of 3,6-anhydrogalactose was calculated.

### 3.7.5.4. Fructose Standard Curve

1ml of D-fructose stock solution was diluted to 10ml to give a concentration of 0.050mg/ml (50$\mu$g/ml). This solution was diluted further in different proportions to yield solutions of 40, 30, 20, 10, and 0$\mu$g/ml. These were then taken through the same experimental procedure at the same time as the samples and a standard curve drawn from the results obtained. Different standard curves were drawn for each set of samples.

### 3.7.6. Sulphate Determination

Sulphur content of the extracts was measured with an electron capture detector coupled to an elemental analyzer (Carlo-Erba 1106) (Kirsten and Nordenmark, 1987). This was done by a commercial company, MicroKemi, Sweden. The procedure involved burning samples in a Carlo Erba 1106 elemental analyzer over copper oxide with oxygen injected as the carrier gas. Combustion gases were then reduced with copper, water absorbed and sulphur dioxide separated from carbon

dioxide and nitrogen in a column of Porapak QS. Sulphur dioxide was then measured with a thermal conductivity detector.

# CHAPTER 4

## A TAXONOMIC REVIEW OF THE GENUS GRACILARIA

The diverse uses of algal phycocolloid required industrial processors to be able to predict properties of algal gels according to their species origin. This process is sometimes hampered by difficulties in identifying species. Hence this study would be incomplete without giving taxonomical details of each species examined.

Connors and Ryther (1985) stated that one of the problems paused to algal taxonomists is the morphological plasticity of the genus Gracilaria. This was further confirmed by McLachlan and Bird (1986) and Bird et al. (1989) who summarised the whole problem by stating that intraspecific taxonomy of the genus is in a chaotic state and is complicated by morphological variability within the species and a paucity of readily apparent phenotypic characters. During an international workshop on the taxonomy of economic seaweeds with reference to the Pacific and Carribean species, it became clear that specimens given the same name differed from region to region hence Chiang et al. (1985) suggested that the keys constructed during the workshop would be more useful if used on geographically limited group of species. Taxonomists are trying to overcome the problem of systematics of the genus through studies encompassing molecular biology, genetics, morphology, anatomy, morphogenesis and sexual hybridization (Bird and Rice, 1990). The

macroscopic morphology, manifested as size and thallus architecture, was the basis for the first species distinction, and this included the morphology of the basal and apical portions of the lateral branches, colour, texture and adherence to the herbarium paper (De Oliveria, 1984). These features are still extensively used (Yamamoto, 1978; 1984) in combination with anatomical and reproductive characters. De Oliveria (1984) has also recommended that within a limited geographical area and based on an adequate number of samples these features can be very useful and in most occasions enough for first identification. For the current study morphological and anatomical features were used to identify the plants and the advice given by Chiang et al. (1985) and de Oliveria (1984) on using these features on geographically limited area would therefore be recommended for the purpose of identifying members of the genus using the observations made in this study. Identification to the genus level was done on fertile specimens using the key developed by Fredericq and Hommersand (1990).

The genus Gracilaria Greville (1830) (Gracilariales, Gracilariaceae) covers some interesting species of red algae from a morphological point of view. Thalli of some species are cylindrical throughout while others are flattened; some are dichotomously branched whereas others are irregularly branched. Some are tough or cartilaginous while others are brittle. All species, however, are parenchymatous and consists of large central cells and a varying development of intermediate cortical cells and

external layer of small pigmented peripheral cells. The central cells range between 400-700microns.

A study of Gracilaria from the Kenya coast has revealed that the following eight species are represented: G. edulis, G. corticata, G. crassa, G. fergusonii, G. millardetii, G. salicornia, G. verrucosa and Gracilaria sp.

The species identified include six of the seven species treated by Jaasund (1976) and two not treated by him. The eight species are part of the 13 species reported by various authors for the country, refer to 4.3. A key to the species, comments on their morphology, illustrations of their growth habit and structure are presented.

**4.1. Taxonomic Key to the Gracilaria species of Kenya**

1. Thallus cylindrical.....2
1. Thallus flattened or partly compressed.....7

2. Thallus prostrate, low growing.....3
2. Thallus erect.....4

3. Thallus fleshy, dichotomously branched with curved top forming bright or dark red tight clumps in pools at the edge of the reef. Turns dark red on drying.....1G. crassa.

3. Thallus dichotomously branched, brittle and forms olive green cushions on the intertidal rocky surfaces and pools. It turns black on drying.....2G. salicornia.

4. Thallus dichotomously or trichotomously branched.....5

4. Thallus irregularly branched ranging from 20cm up to 1.5m long and thin thus having a filamentous appearance. The very long ones grow prostrate on sand hence partly covered by sand. The thallus is cartilaginous and has numerous proliferations. It is dark red or straw coloured.....3G. verrucosa.

5. Thallus between 0.5 and 1.5mm in diameter, dichotomously branched, rigidly cartilaginous and dark red, measuring between 7 and 10cm in length.....4G. edulis.

5. Thallus ranging from 1-4mm in diameter, brittle or fleshy, either dichotomously or trichotomously branched.....6

6. Thallus dichotomously branched short 4-10cm long, smooth and fleshy, top branching and reddish brown.....5G. fergusonii.

6. Thallus 7-25cm long, brittle, olive green with smooth

margins. At times it has constrictions that give it a beaded appearance; they are either dichotomously or trichotomously branched .....2G. salicornia.

7. Thallus dichotomously branched.....8

7. Thallus irregularly branched, completely flattened, cartilaginous , 3-7cm long with frequent projections on the margins. Colour of thallus is dark red and turns black on drying.....
.....6G. millardetii.

8. Thallus completely flattened, cartilaginous with forked rounded tips and smooth margins. Live specimens are brownish yellow and turn red on drying.....7Gracilaria sp.

8. Thallus completely flattened or partly compressed, cartilaginous, grows either epiphytically on seagrasses with the help of rhizoidal structures or on rocks with a holdfast. Branching is primarily dichotomous though numerous secondary branchlets and projections make it look irregular especially at old age. Colour is dark red and turns black on drying.....8G. corticata.

**4.2. Species Morphological Descriptions and Illustrations**

G. crassa Harvey.

Original description by Harvey (1859).

Voucher Numbers: 1002 - collected on 14-9-89 at Fort Jesus

from eulittoral zone.

1021 - collected on 20-8-90 at Mombasa Hospital from eulittoral zone.

1058 - collected on 17-12-91 at Tiwi from sublittoral zone.

This is a species that grows directly on rocks with a rhizoidal holdfast. The thalli branch dichotomously with curved top thus forming very thick fleshy and smooth cushions that break easily on removal. The cylindric bright red thalli turn dark red on drying and their sizes range between 3 - 6cm in length with a diameter of 2 - 4 mm and no main axis is distinguishable. Plate 5.

G. salicornia (J. Ag.) Dawson.

Original description by Agardh (1852) and revised by Dawson (1949).

Voucher Numbers: 1009 - collected on 2-11-89 at Gazi from sublittoral zone.

1019 - collected on 12-11-89 at Gazi from sublittoral zone.

1039 - collected on 2-7-91 at McKenzie point from eulittoral zone.

1040 - collected on 2-7-91 at McKenzie point from eulittoral zone.

The species is cylindrical, succulent and dichotomously or trichotomously branched with smooth margins, and the branching is lax. Trichotomy in the branching pattern is more frequently observed on specimens that are sublittoral. The specimens range between 7 and 25 cm long with a diameter of between 1 and 4 mm depending on the habitat. At times the thalli have constrictions that give it a segmented appearance. The colour of the fresh specimens range from yellowish green to olive green and these turn black on drying. The thalli grow as cushions on exposed rocky surfaces or grow erect in sublittoral conditions and intertidal pools. The cushions form caespitose clumps that can be as big as 20cm wide on muddy-rocky intertidal surfaces while on bare rocky surfaces they are continuous. The plants that grow erect have distinguishable main axis while this is not the case in the decumbent type. Plates 6-8.

G. verrucosa (Huds.) Papenfuss

Original description by Papenfuss (1953).

Voucher Numbers: 1013 - collected on 23-7-89 at Gazi from the sublittoral zone.

1018 - collected on 17-7-90 at Vanga from the sublittoral zone.

1019 - collectted on 17-7-90 at Vanga from the sublittoral zone.

1041 - collected on 17-7-91 at Gazi from the sublittoral zone.

The species is cylindric, long and thin thus resulting in a filamentous appearance. The thalli are anything from 20cm to 1.5m long and have a diameter of between 0.2 to 1mm depending on the part of the thallus. The branching pattern is irregular with numerous branchlets coming off from the major branches with no distinguishable main axis. The colour of the species is either that of straw or dark red. The fact that they are long and thin make the very long ones lie prostrate to the sand thus having parts of the thallus embedded in sand. Plate 9.

G. edulis (J. Ag.) Silva

Original description by Agardh (1852) and revised by Silva (1952).

Voucher Numbers: 1022 - collected on 4-7-91 at Wasini from eulittoral zone.

1035 - collected on 21-1-88 at Kanamai from eulittoral zone.

1051 - collected on 2-7-91 at McKenzie point from eulitteral zone.

The species has a dark red cylindrical thallus that has primarily a dichotomous branching pattern. It is rigidly cartilaginous and the specimens observed ranged between 7 and 10cm in length and had a

thickness of between 0.5 - 2mm depending on the part of the thallus. The thallus has a small discoid holdfast for attaching itself onto small pieces of rocks and there is a distinct main axis on the lower parts. Plate 10.

G. fergusonii J. Agardh

Original description by Agardh (1852).

Voucher Numbers: 1015 - collected on 4-7-91 at Wasini Island from sublittoral zone.

1037 - collected on 14-7-90 at Nyali beach from eulitteral zone.

1057 - collected on 5-6-90 at Wasini from sublittoral zone.

The species exhibits clear dichotomy in its branching pattern with a distinct main axis which branches mainly towards the top. The thalli were often observed growing solitarily. It has a reddish brown colour which turns dark red on drying. The thalli are succulent, cylindric, 4 - 8 cm long and with a diameter of 2 - 3mm depending on the size of the specimen. Plate 11.

G. millardetii J. Agardh

Original description by Agardh (1852).

Voucher Numbers: 1001 - collected on 16-10-89 at Fort Jesus from eulittoral zone.

1038 - collected on 20-8-90 at Fort Jesus from eulittoral zone.

1052 - collected on 19-7-91 at Fort Jesus from eulittoral zone.

The plants have thalli that are flat and branch irregularly and profusely thus appearing bushy with no discernable main axis. They are cartilaginous, dark red when fresh and turn black on drying. The size of the specimens vary between 4 and 8 cm long while the blades have a diameter of 0.5 - 3mm depending on the position of the branch. There is frequent formation of short and long projections forming teeth-like structures on the margins and these add to the irregularity of the branching pattern. The species are attached to rocks by a small discoid holdfast. Plate 12.

Gracilaria. sp. nov.

Voucher Numbers: 1010 - collected on 30-11-89 at Mambrui

from eulittoral zone.

1032 - collected on 20-8-90 at Mombasa Hospital from eulitteral zone.

1045 - collected on 19-3-91 at Mambrui from eulittoral zone.

1053 - collected on 27-5-91 at Mambrui from eulittoral zone.

1055 - collected on 23-7-91 at McKenzie point from eulittoral zone.

1056 - collected on 14-8-91 at Mombasa Hospital from eulittoral zone.

The thallus is cartilaginous, flat, 3 - 6 cm long and with a blade diameter of 2 - 4 mm broad. The pattern of branching is dichotomous with the main axis distinguishable from the lower parts. The margins are smooth and tips rounded and bi-lobed. The colour of fresh specimens is brownish yellow and pinkish red when dried. It has rhizoidal structures for attachment onto the rocks. Plate 13.

G. corticata J. Agardh

Original description by Agardh (1852).

Voucher Numbers: 1005 - collected on 23-10-89 at McKenzie point from sublittoral zone.

1008 - collected on 2-11-89 at Gazi from sublittoral zone.

1011 - collected on 30-11-89 at Mambrui from sublittoral zone.

1012 - collected on 30-11-89 at Mambrui from eulittoral zone.

1020 - collected on 17-7-89 at Vanga from eulittoral zone.

1033 - collected on 21-8-90 at McKenzie point from eulittoral zone.

1037 - collected on 21-8-90 at McKenzie point from eulittoral zone.

The species has partly compressed or completely flattened thalli that are cartilaginous and branch dichotomously. The dichotomy during fertility is marred by lots of ramifications that give them an irregular pattern. The colour of fresh specimen is dark to purplish red and on drying they turn black. The sizes of the thalli range between 6 - 20 cm

in length and 1 - 4 mm wide depending on the habitat. The population observed in the sandy channels posses narrow, long, tough and thick blades which are heavily intertwined.

The species grows either as an epiphyte on seagrass (Thalassodendron. ciliatum), in association with Halodule or uses rhizoids to grow directly on rocks. No main axis is distinguishable in this species as several thalli grow off from the basal parts. Plates 14-16.

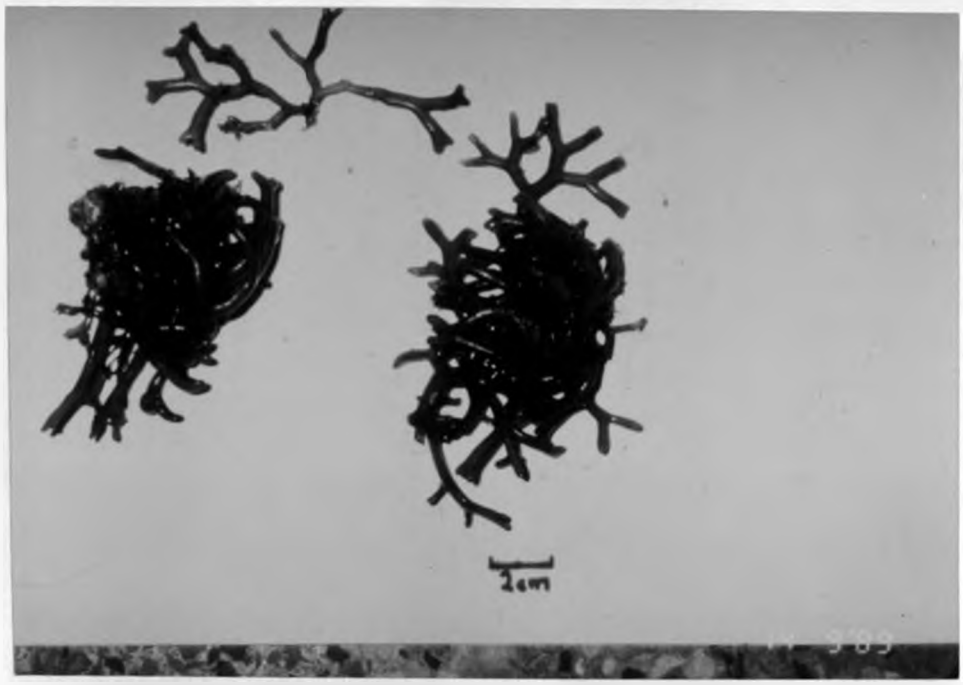



Plate 5. Habit of G. crassa

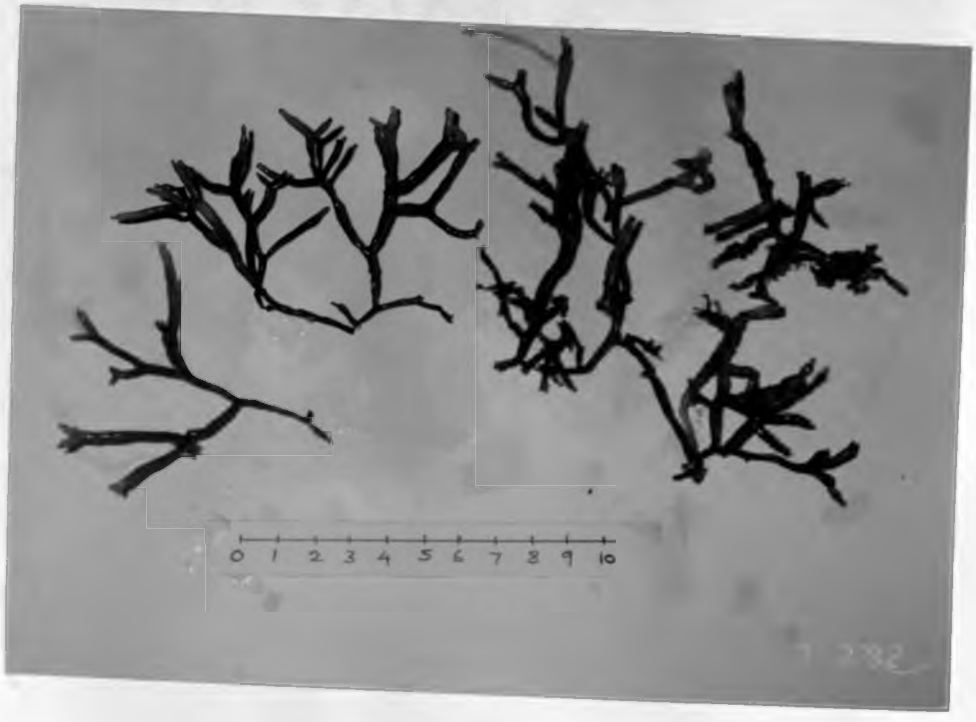



Plate 6. Habit of G. salicornia intertidal type.

Plate 7. Habit of G. salicornia sublittoral type.


Plate 8. Habit of G. salicornia cushion forming type.

Plate 9(a). Habit of G. verrucosa (young)

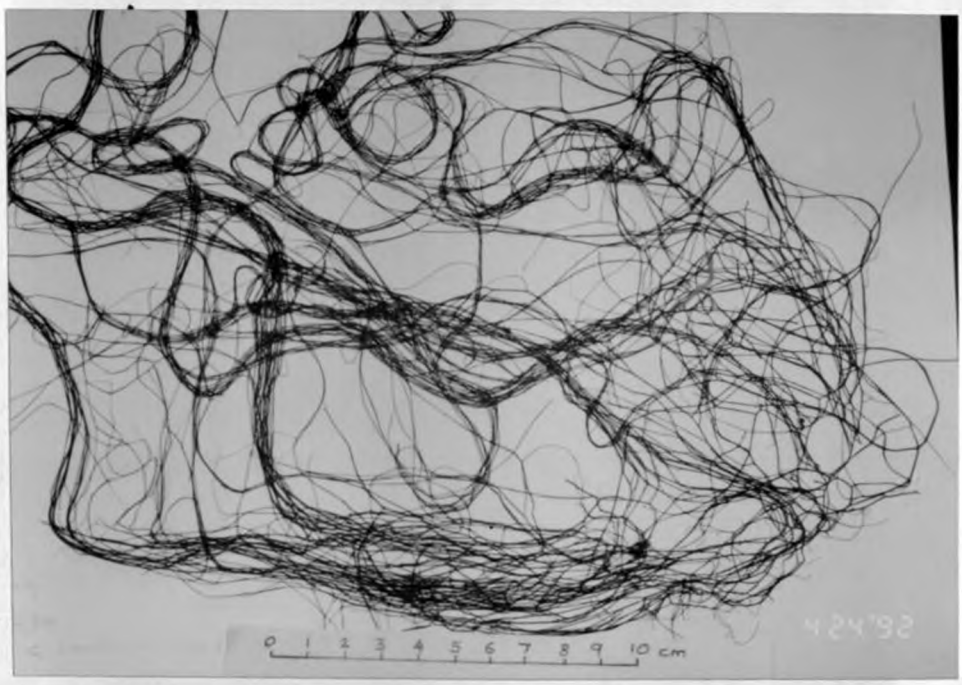



Plate 9(b). Habit of G. verrucosa (mature)

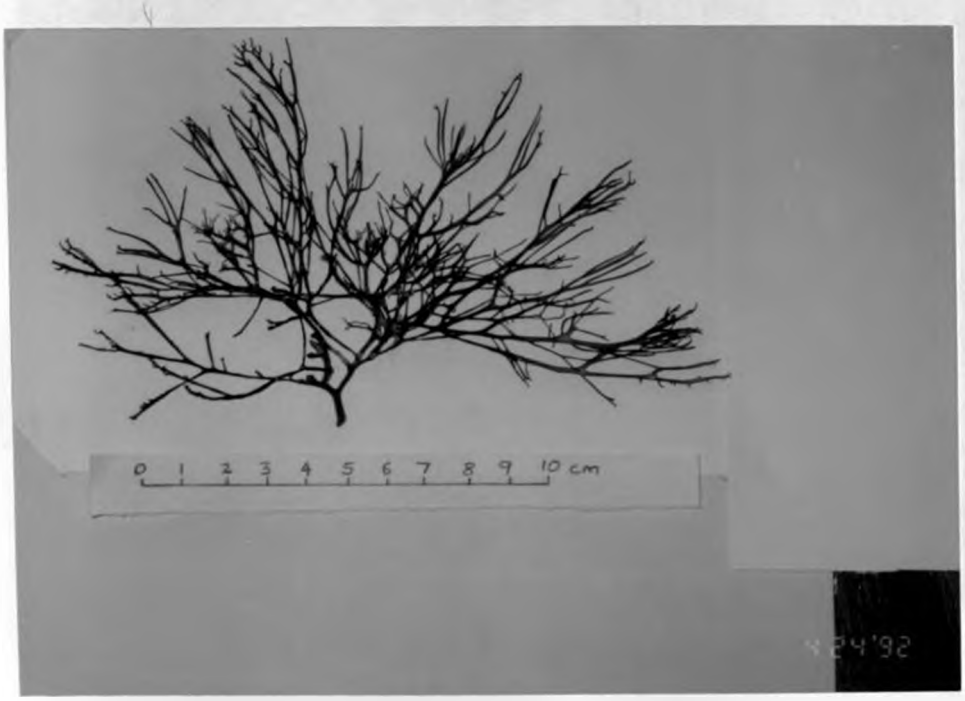



Plate 10. Habit of G. edulis


Plate 11. Habit of G. fergusonii

Plate 12. Habit of G. millardetii

Plate 13. Habit of Gracilaria sp.

Plate 14. Habit of epiphytic

G. corticata

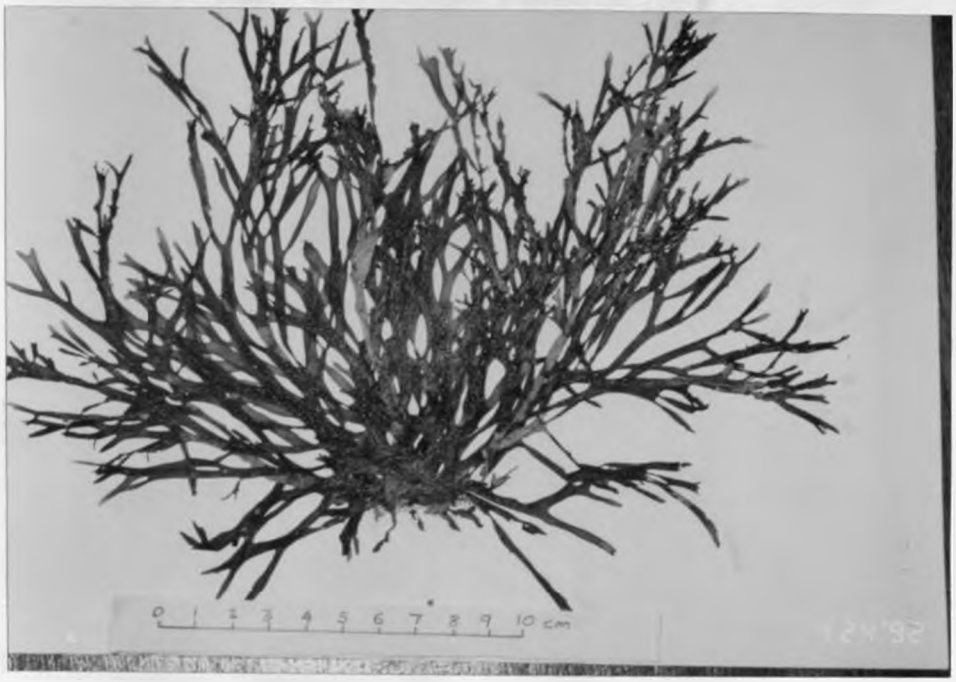



Plate 15. Habit of rocky type

G. corticata

Plate 16. Habit of sandy type

G. corticata

## 4.3. A Revision on Previous Reports on Kenyan Gracilaria

Work done by Isaac, voucher specimens deposited at the herbarium section of the Kenya National Museum, and Moorjani (1977) suggested that there are a total of 13 Gracilaria species in Kenya i.e. G. bursapastoris, G. cacalia, G. corticata, G. crassa, G. dura, G. edulis, G. fergusonii, G. foliifera, G. lichenoides, G. millardetii, G. purpurascens, G. salicornia, G. verrucosa. Current study has shown that 6 of the species reported were misidentified. Similar specimens were collected and their morphological and anatomical features studied. Misidentification of specimens could have been due to morphological variability which, as Chapman et al. (1977) reported, may be so great as to have individual species being known by several names. The misidentified specimens are as follows:-

G. bursa-pastoris

Isaac Voucher Number (V.N) 3212

Morphological and anatomical features are that of Soliera. Oyieke V.N.1007

G. cacalia

Isaac V.N. 2829, 2359 and 4707 have features of G. salicornia.

Oyieke V. N. 1019.

Isaac V. N. 2299 and 3520 have features of G. crassa, Oyieke V. N. 1002, 1021.

G. dura

Isaac V. N. 4501 have features of Hypnea. Oyieke V. N. 1049.

G. foliifera

Isaac V.N. 2071, 4370, 3871, 3326 have features of Sarcodia, Oyieke V. N. 1004.

G. lichenoides

Isaac V.N. 2026 have features of Sarconema. Oyieke V. N. 1047.

Isaac V.N. 2452 have features of G. edulis. Oyieke V. N. 1022.

G. purpurascens

Isaac V.N. 3861 have features of Sarcodia. Oyieke V. N. 1004.

Isaac V.N. 3862 have features similar to G. fergusonii sp., Oyieke V. N. 1015, 1037, 1057.

Table 1: Gracilaria species as recognized in the study and their synonyms in Kenyan previous records.

| Gracilaria species as recognized in this study | Synonyms from previous records |
|---|---|
| G. crassa | G. cacalia (J.Ag.) Dawson |
| G. salicornia | G. cacalia (J.Ag.) Dawson |
| G. verrucosa | - |
| G. edulis | G. lichenoides (Linne) Harvey |
| G. fergusonii | G. purpurascens Harvey |
| G. millardetii | - |
| G. corticata | G. millardetii J. Agardh |
| Gracilaria sp. nov. | - |

One species reported by the above authors, G. arcuata has not been studied since specimen materials were not found. From this study it has become evident that morphological descriptions and illustrations of individual species have variations that depend on the type of habitat that each specimen is found in. The species that have been observed to exhibit a great deal of morphological variations are, G. salicornia and G. corticata. Morphological variability in G. salicornia has also been reported by other workers (Abbott, 1986; Meneses and Abbott, 1987) and several species in China have recently been designated as its synonyms (Abbott, 1986)

Xia (1986) observed that features chosen to distinguish G. salicornia from the Carribean and Pacific region were not constant and that the same features were supposed to identify G. crassa, G. cacalia, G. minor and G. canaliculata. The placement of some specimens of G. crassa and G. salicornia under G. cacalia by Isaac is therefore not a unique occurrence to the Kenyan species. Of the Kenyan specimens the morphological features that have consistently distinguished G. salicornia from G. crassa is the colours and texture of the thalli. Whereas G. salicornia is succulent and brittle, G. crassa is fleshy and firm. The former has shown its colour to vary from greenish yellow to olive green

while the latter is either dark or bright red.

Though it has been repeatedly stated that systematics within the genus Gracilaria is in a "chaotic" state it is important that a system is worked out for identifying specimens within a region. Reliable all-round indicators are needed which are visible regardless of whether the fronds are young or old. The most effective and consistent feature to divide Kenyan species into two large groups is whether the fronds are flattened or cylindrical in cross sectional view. Specimens of G. corticata, G. millardetii and Gracilaria sp. are all flattened or partly compressed regardless of their habitats and microhabitats while those of G. crassa, G. edulis, G. fergusonii, G. salicornia and G. verrucosa are all cylindrical. However, this feature may not be useful universally as variations have been reported in some species (May, 1948).

The use of colour as an identification feature may not be very useful on its own, since this has been found to vary with habitats. Nonetheless, Gracilaria sp. has been observed to be very consistent in its colour of brownish yellow which can easily make one mistake it for a phaeophyte. The branching pattern is another feature that can be used quite reliably. All the specimens of G. crassa, G. corticata, G. edulis, G.

fergusonii. and Gracilaria sp. exhibit dichotomy in their branching pattern though this is difficult to recognise in G. corticata at its advanced stages of development when it has numerous ramifications which give it an irregular appearance on first sight. G. salicornia on the other hand, is the only species which was observed to exhibit dichotomy as well as trichotomy in its branching pattern. All specimens of G. millardetii and G. verrucosa branch irregularly.

With all the above described features it should be possible to identify the species in this study with ease. However, due to the variations reported, it should be stressed that the use of morphological and phenotypic features in identifying species of the Kenya coast should go hand in hand with information regarding the ecological background of each specimen.

# CHAPTER 5

# THE ECOLOGY AND DISTRIBUTION OF GRACILARIA

Though much work has been done on the ecology of the genus in different parts of the world, information on the Kenyan species is virtually lacking. Just as taxonomic studies are important for the purpose of correct identification, similarly ecological studies are important for the purpose of identifying the habitats that are associated with the best strains for industrial purposes.-

## 5.1. Ecological Habitats

During this study it became evident that different Gracilaria species require different habitats for optimal growth and hence an attempt has been made to describe the ecological habitats of the various species. The species studied can broadly be categorized into 2 groups:-

(a) the eulittoral (intertidal) species and

(b) the sublittoral species.

Most of the shores where investigations were carried out are nearly all rocky, made of coral limestone and consisting of steep cliffs stationed at the landward edge of the reef flats. Some of the flats have

sandy beaches at the landward edge instead of cliffs. The reef flats are erosion platforms which have been cut into raised coral limestone. These platforms are of variable widths as the profiles of the study sites indicate (Figure 18 a-v). There are living corals at the outer edge of the reefs though occasionally some are found in the inner lagoons. The main surface of the flats is eroded limestone, with occasional pools, patches of sand and cast up coral boulders. There is a mixed flora of algae and seagrasses on the platforms. The platforms slope gently upwards to about mean tide waters or a little higher at which level the cliffs arise abruptly. These limestone cliffs are of varying heights. The stations studied have cliffs ranging between 4 and 1m above sea level. The cliffs are steep and often have heavy undercuts which are typical features of limestone cliffs in the tropics. A description of the various microhabitats where different Gracilaria species were encountered is as follows:

(a) Rocky Pools

These are pools within the reef platform and they are rocky throughout including their bottoms. Their sizes range from 20cm to 2m in diameter and with a depth of 10 - 50cm.

(b) Sandy Pools

These are also found within reef platforms and have similar dimensions as for the rocky pools. The only difference is that they have purely sandy bottoms.

(c) Lagoons

Within some reef platforms are large expanses of water. Their diameters are from 2 to 100m while their depths are over 0.5m. They are permanently filled with water whether it is low or high tide.

(d) Muddy shores

These shores are found where there are mangrove forests and a few meters beyond. They consist of fine dark mud that gradually give way to sandy shores.

(e) Rocky platforms

Within the reef platform are areas that have exposed rocky surfaces, some totally bare while others covered by a thin film of sand.

(f) Channels

These are bodies of moving water, whose direction of flow depend on whether the tide is ebbing or flooding. The bottom of these channels is either sandy or muddy, or a mixture of rock and sand.

(g) Reef Edge

This is the edge of the reef platform. It often consists of large rocks and boulders forming several rocky pools and hidden crevices. This is the roughest part of the platform in terms of wave action as the waves break here.

All the Gracilaria species considered sublittoral in this study are those that are covered by water even at low water spring. The bottom topography of these sublittoral habitats vary from seagrass beds to rocky bottoms with dispersed sandy patches, whereas others are purely sandy.

Figure 16 (a and b) show the distribution of the stations studied along the Kenya coast while the profiles of all the stations studied, showing details of different habitats are shown in figure 18 (a-v). Table 1 shows the distribution of the eight species of Gracilaria along the coast.

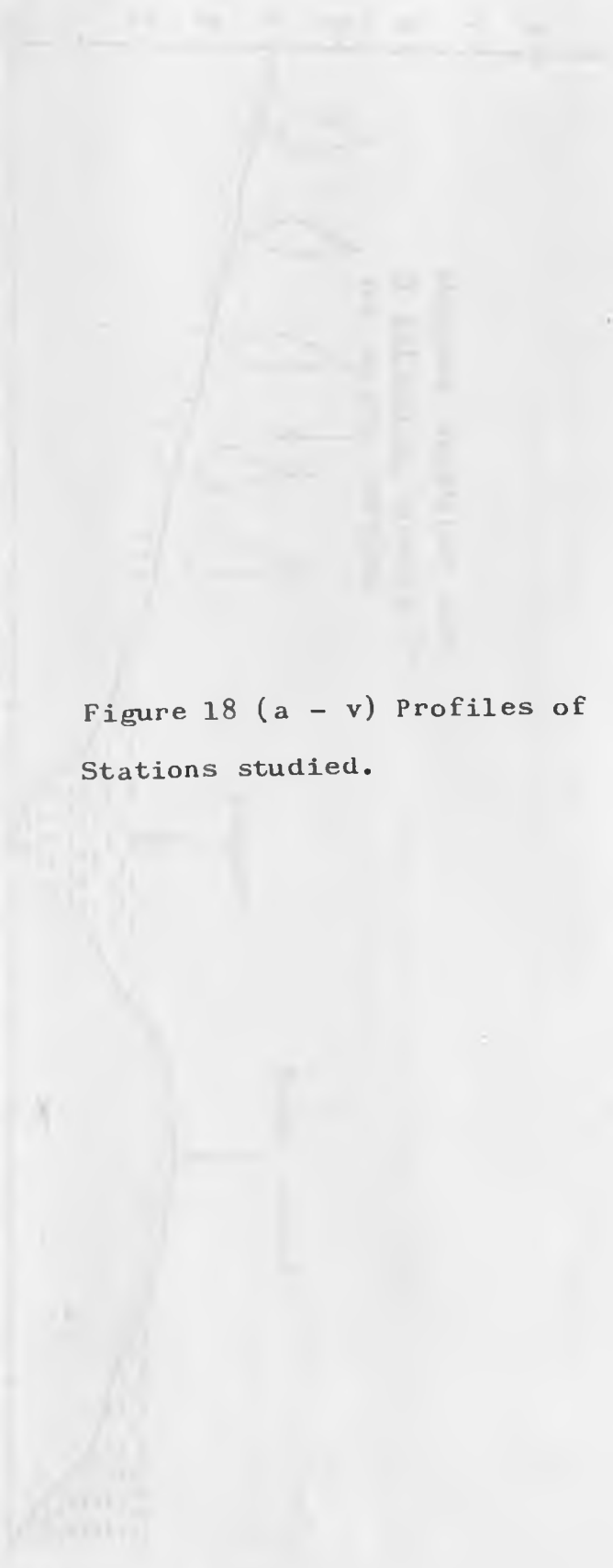
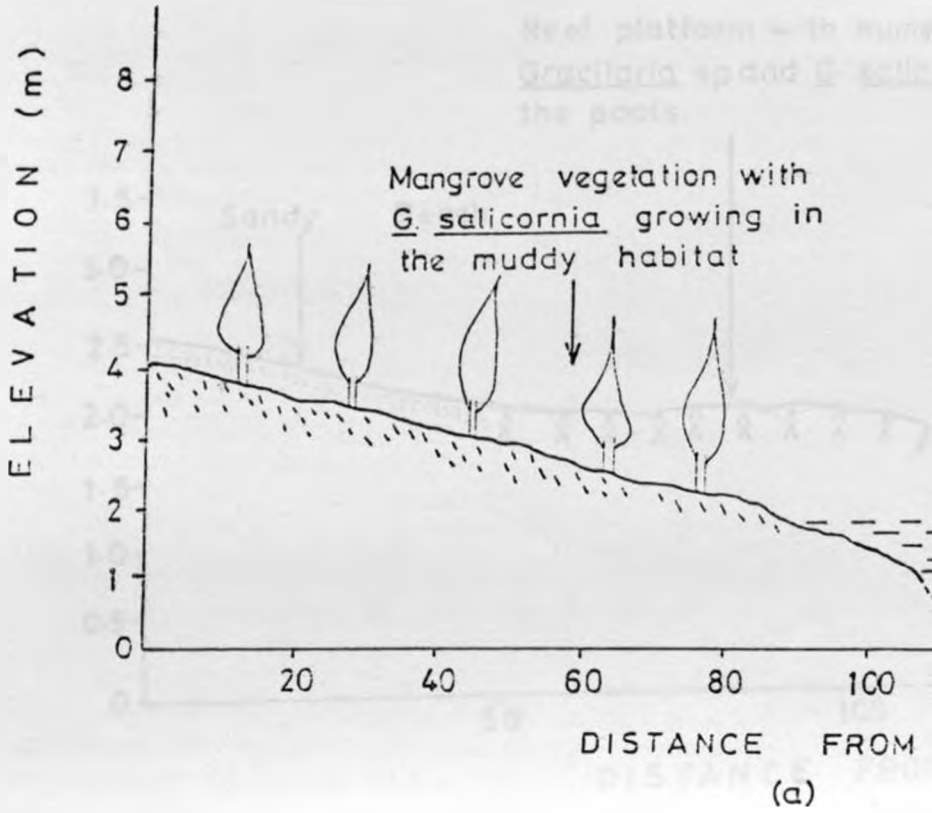
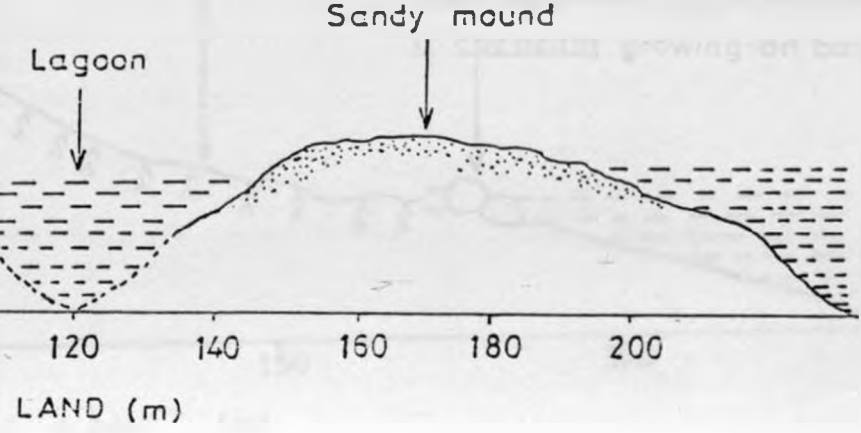


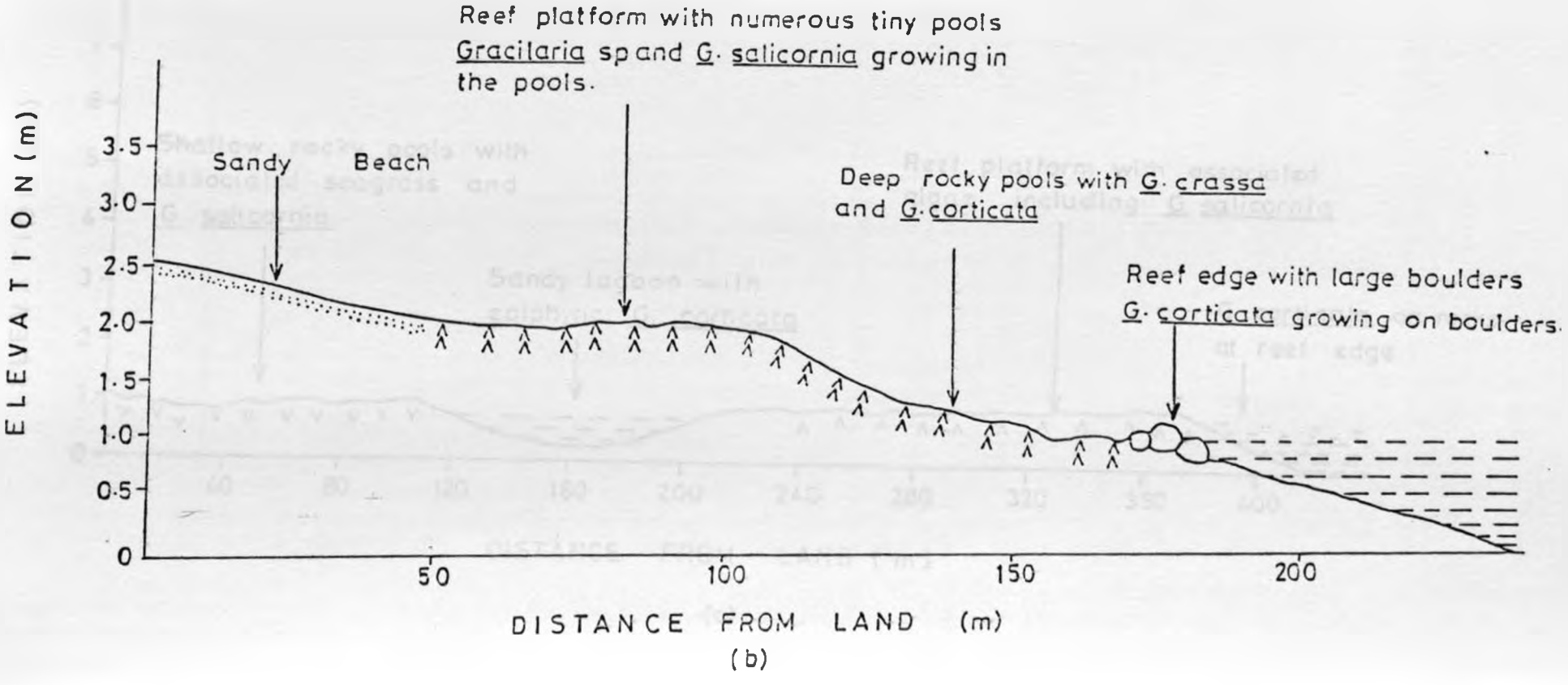
Figure 18 (a - v) Profiles of Stations studied.

NGOMENI

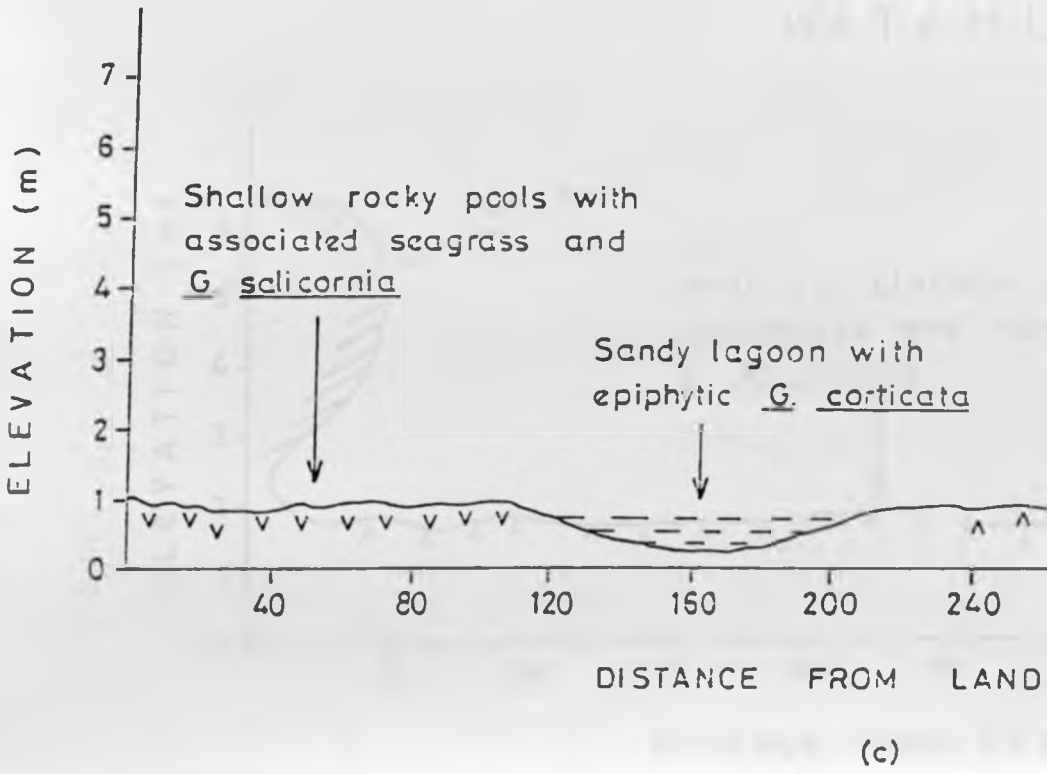

ELEVATION (m)
8
7
6
5
4
3
2
1
0
Mangrove vegetation with
G. salicornia growing in
the muddy habitat
20
40
60
80
100
DISTANCE FROM
(a)


Sandy mound
Lagoon
120
140
160
180
200
LAND (m)

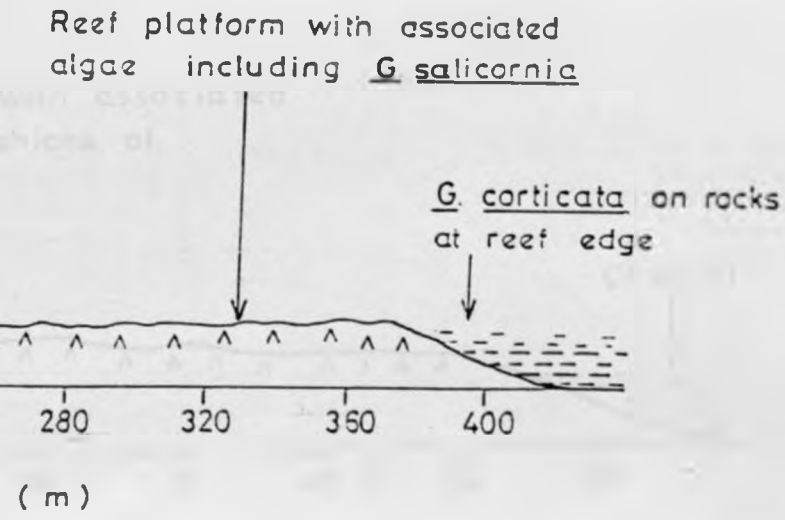
# MAMBRUI

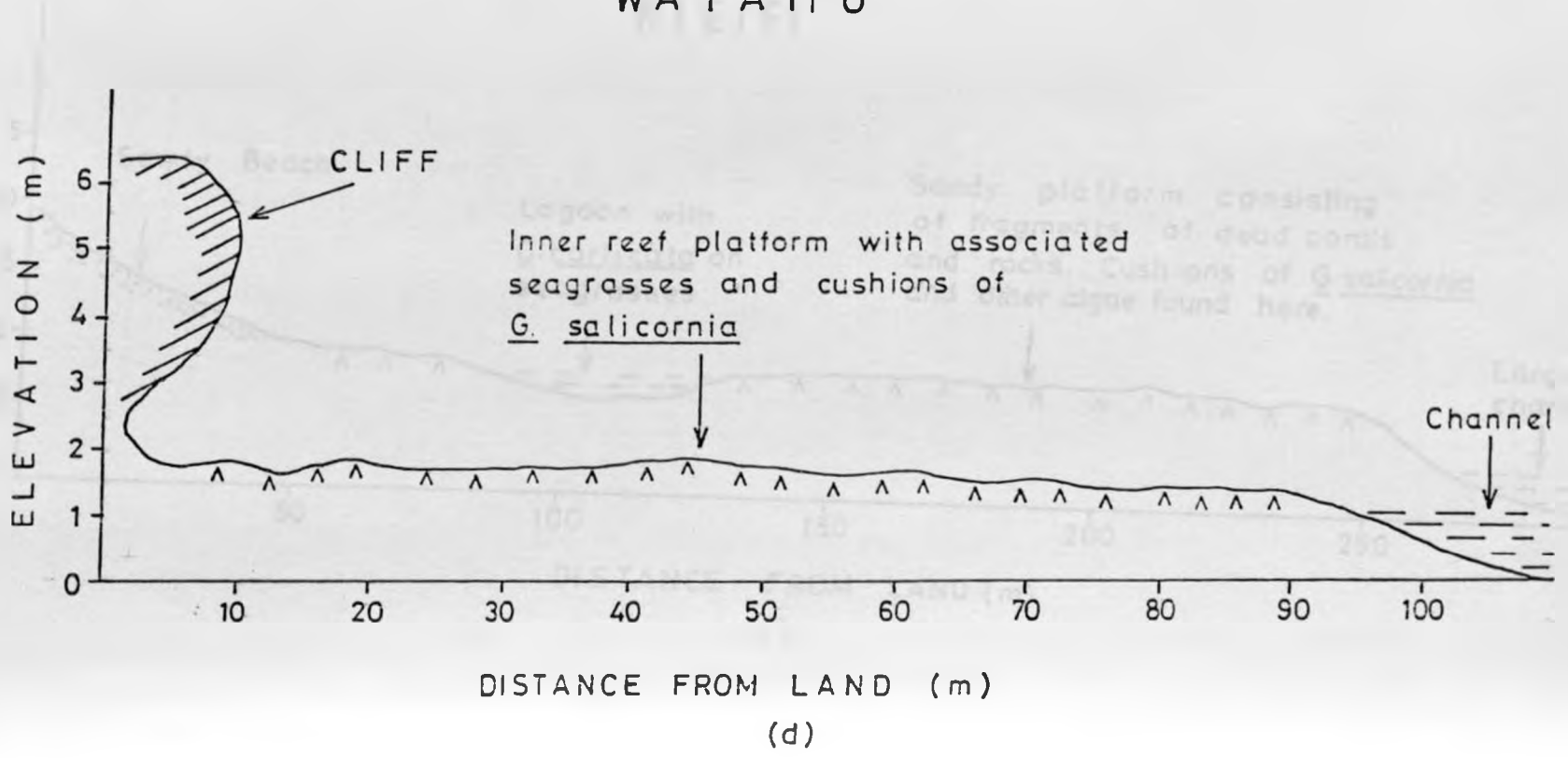

Reef platform with numerous tiny pools
Gracilaria sp and G. salicornia growing in the pools.
Sandy Beach
Deep rocky pools with G. crassa and G. corticata
Reef edge with large boulders
G. corticata growing on boulders.
ELEVATION (m)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
50
100
150
200
DISTANCE FROM LAND (m)

(b)

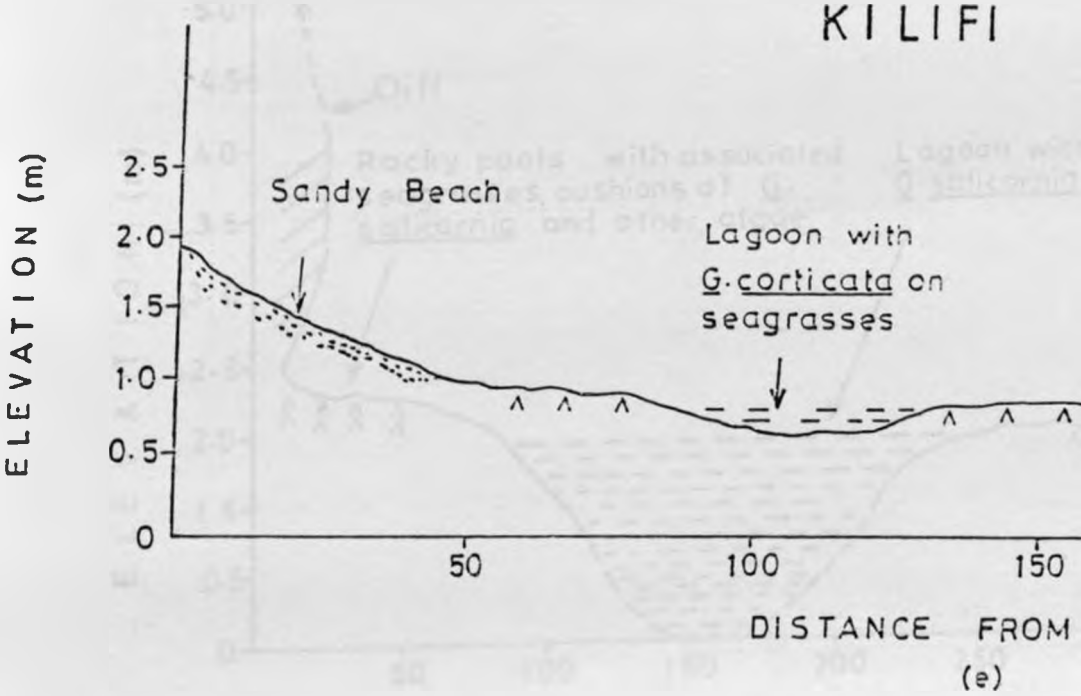

MALINDI
ELEVATION (m)
7
6
5
4
3
2
1
0
Shallow rocky pools with associated seagrass and G. salicornia
Sandy lagoon with epiphytic G. corticata
40
80
120
160
200
240
DISTANCE FROM LAND

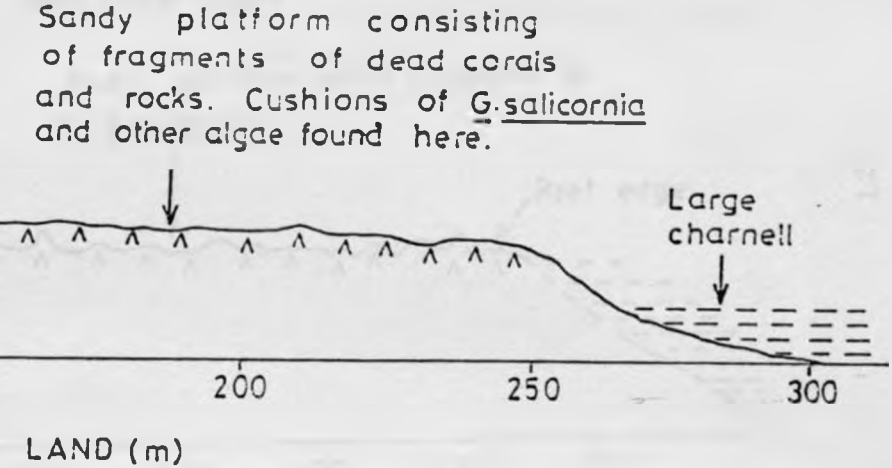
(c)


Reef platform with associated algae including G salicornia
G. corticata on rocks at reef edge
280
320
360
400
( m )

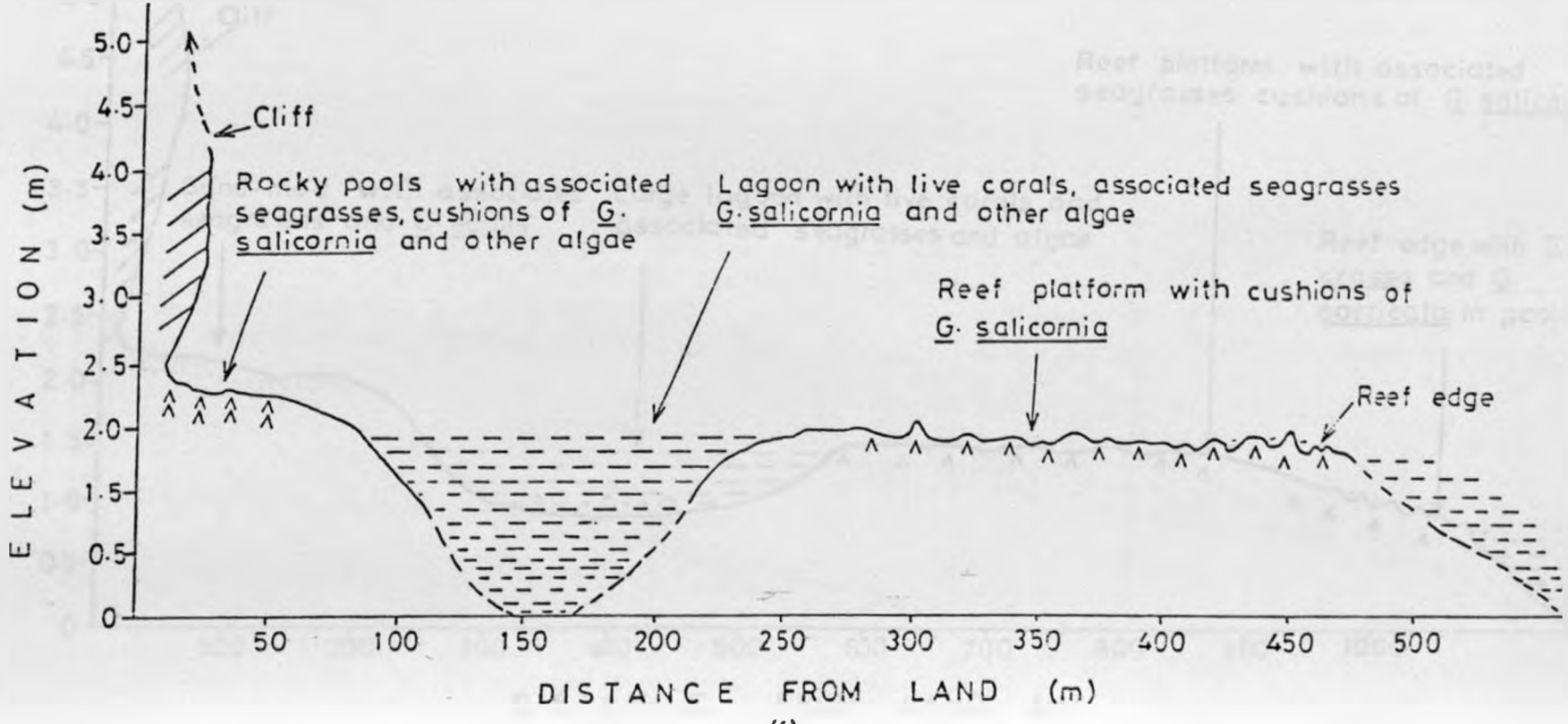

WATAMU
CLIFF
Inner reef platform with associated seagrasses and cushions of G. salicornia
Channel
ELEVATION (m)
0
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
100
DISTANCE FROM LAND (m)

(d)

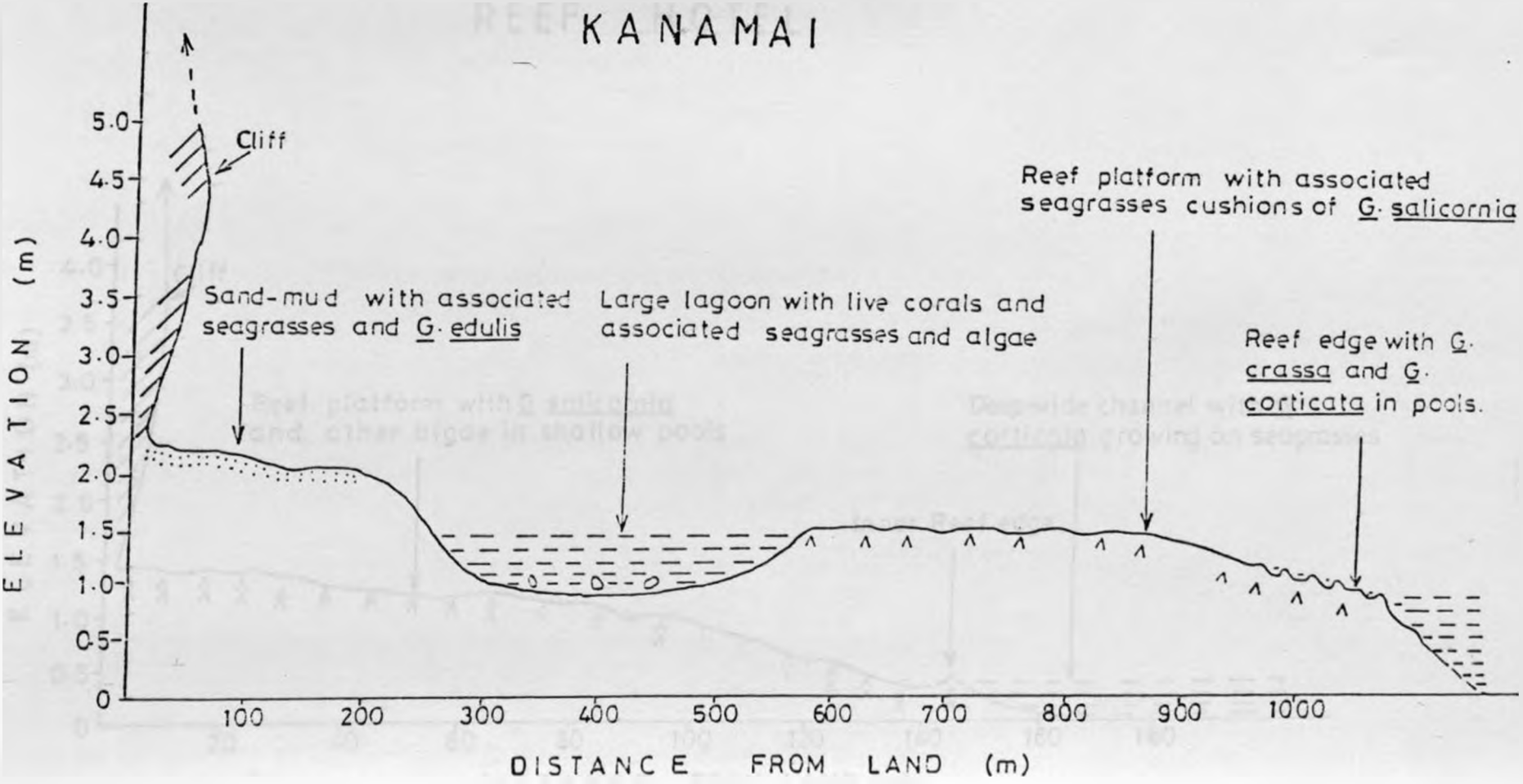

KILIFI
ELEVATION (m)
2·5
2·0
1.5
1·0
0.5
0
Sandy Beach
Lagoon with
G.corticata on
seagrasses
50
100
150
DISTANCE FROM
(e)


Sandy platform consisting of fragments of dead corals and rocks. Cushions of G. salicornia and other algae found here.
Large charnell
200
250
300
LAND (m)

# VIPINGO

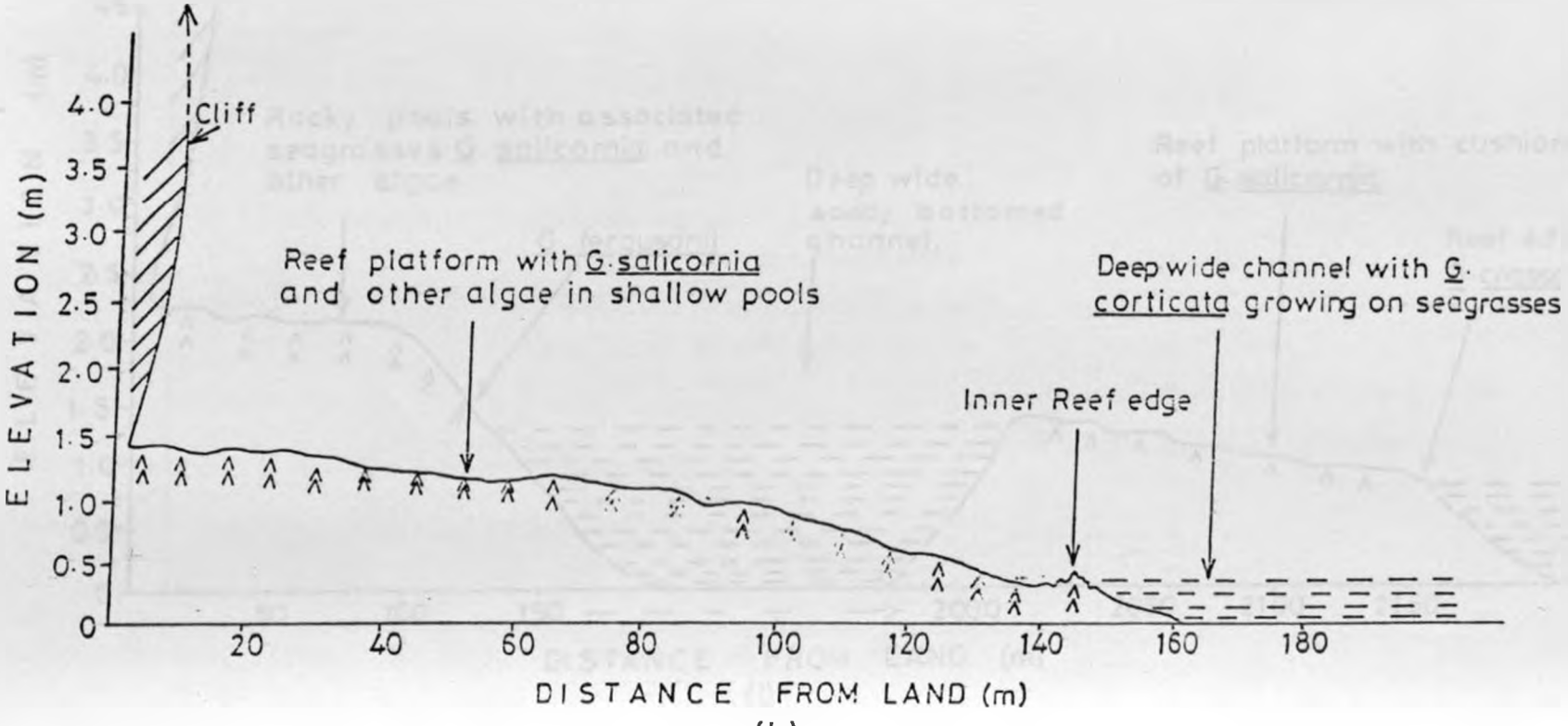

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0.5
0
ELEVATION (m)
Cliff
Rocky pools with associated seagrasses, cushions of G. salicornia and other algae
Lagoon with live corals, associated seagrasses G. salicornia and other algae
Reef platform with cushions of G. salicornia
Reef edge
50
100
150
200
250
300
350
400
450
500
DISTANCE FROM LAND (m)

(f)

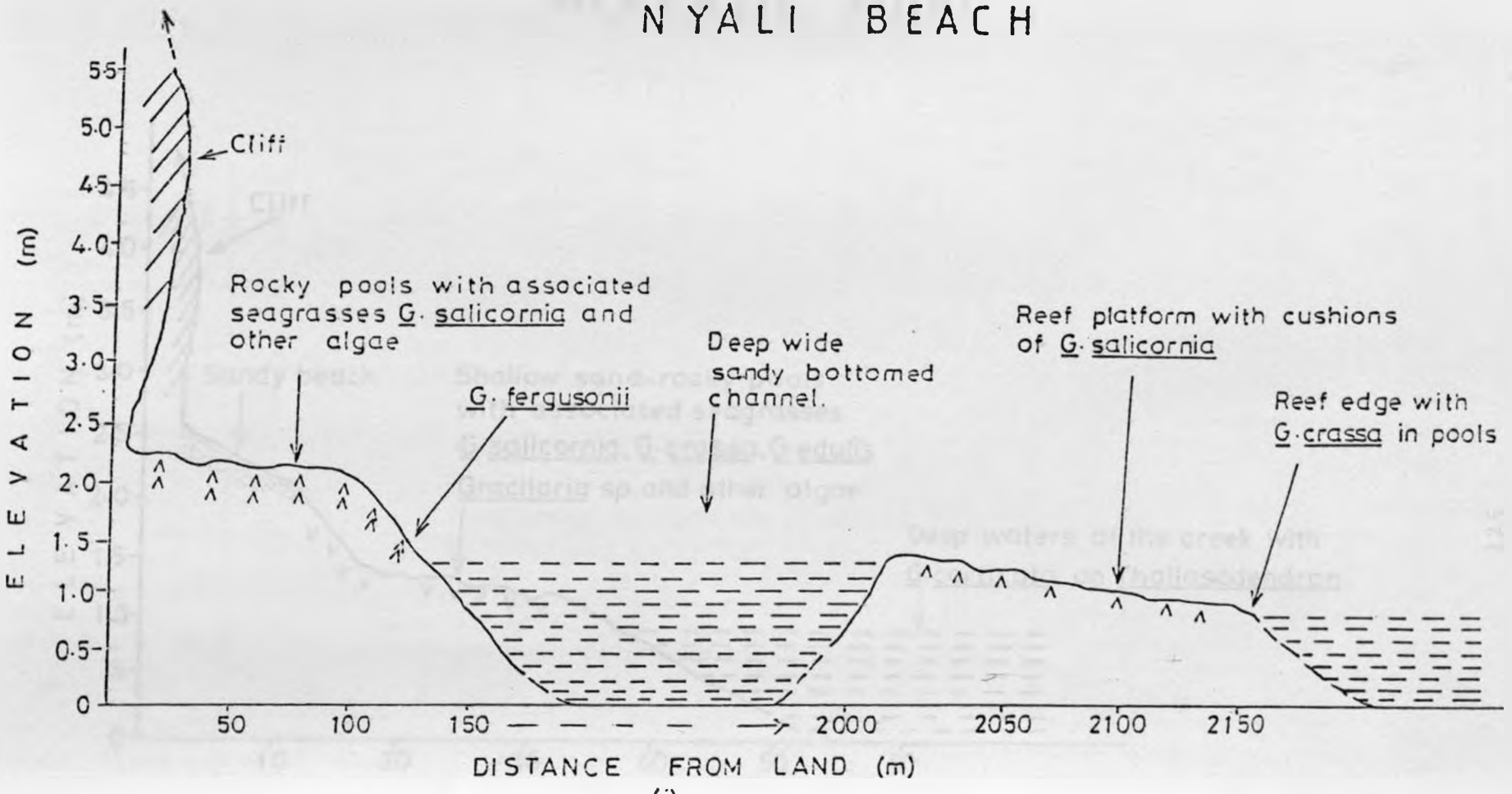

KANAMAI
Cliff
Sand-mud with associated seagrasses and G. edulis
Large lagoon with live corals and associated seagrasses and algae
Reef platform with associated seagrasses cushions of G. salicornia
Reef edge with G. crassa and G. corticata in pools.
ELEVATION (m)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
100
200
300
400
500
600
700
800
900
1000
DISTANCE FROM LAND (m)

(g)

# REEF HOTEL

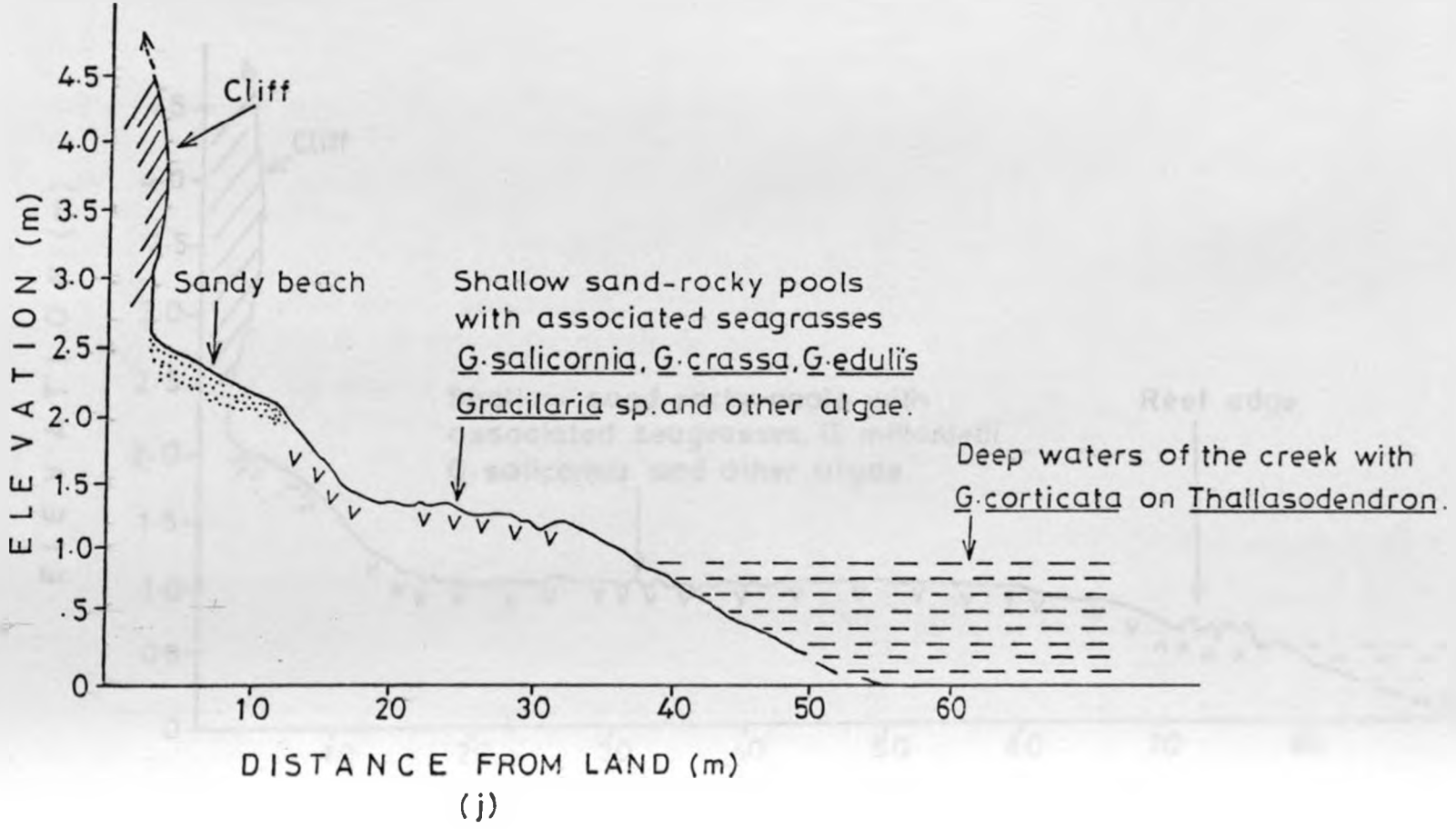

Cliff
Reef platform with G. salicornia and other algae in shallow pools
Deep wide channel with G. corticata growing on seagrasses
Inner Reef edge
ELEVATION (m)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
20
40
60
80
100
120
140
160
180
DISTANCE FROM LAND (m)

(h)


NYALI BEACH
ELEVATION (m)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Cliff
Rocky pools with associated seagrasses G. salicornia and other algae
G. fergusonii
Deep wide sandy bottomed channel.
Reef platform with cushions of G. salicornia
Reef edge with G. crassa in pools
50
100
150
2000
2050
2100
2150
DISTANCE FROM LAND (m)

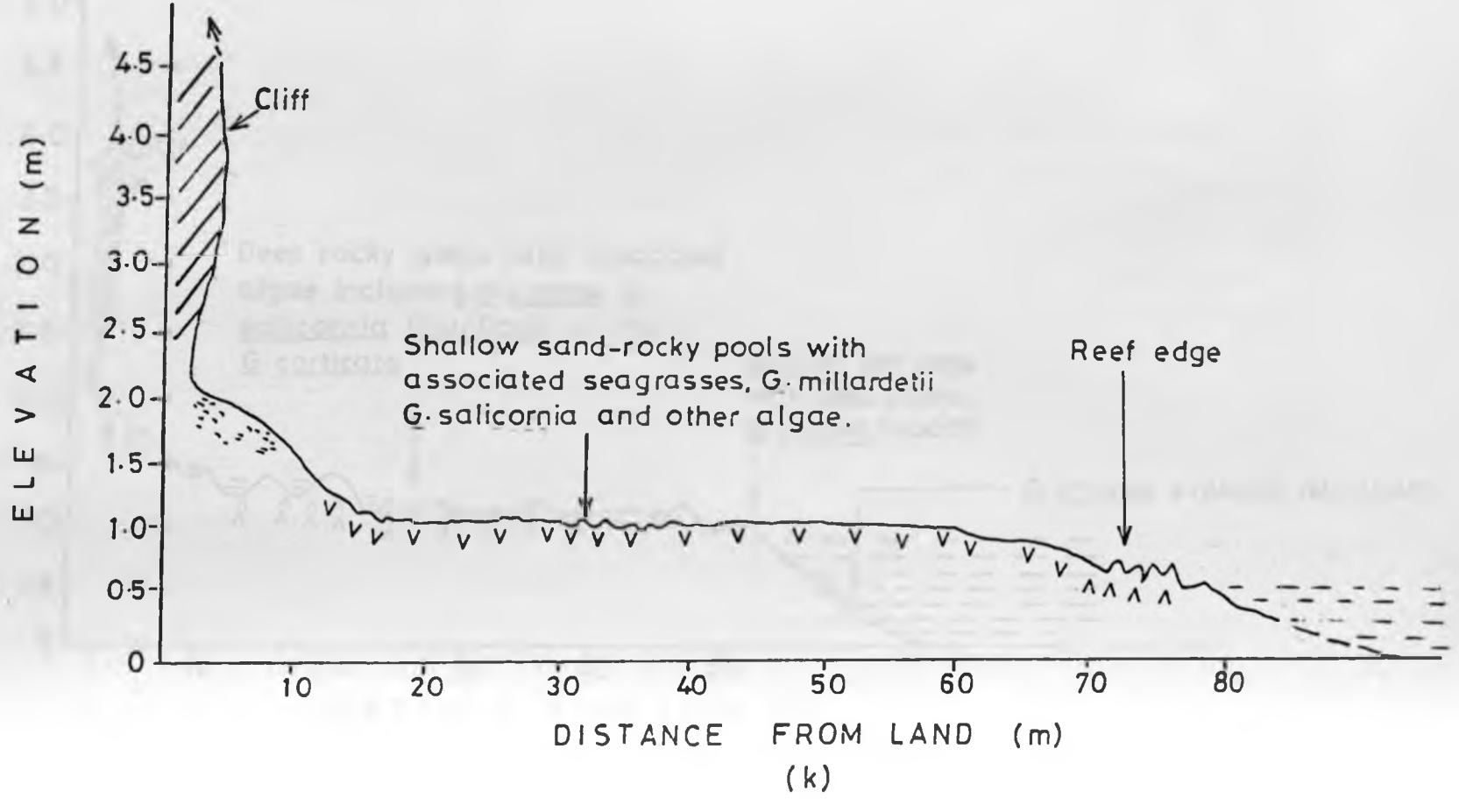
(i)

# MCKENZIE POINT



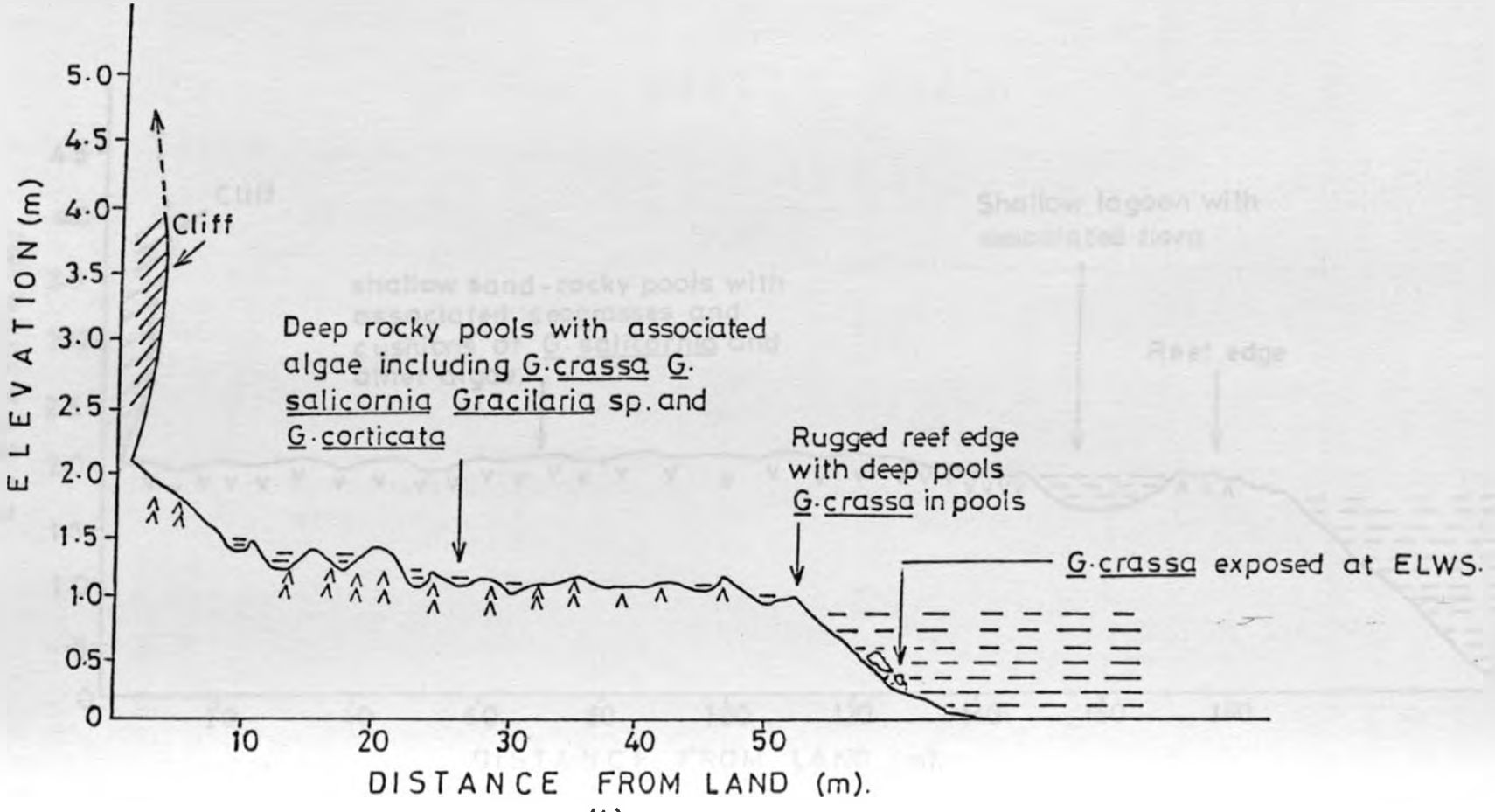

(j)

# FORT JESUS


ELEVATION (m)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Cliff
Shallow sand-rocky pools with associated seagrasses, G. millardetii G. salicornia and other algae.
Reef edge
10
20
30
40
50
60
70
80
DISTANCE FROM LAND (m)

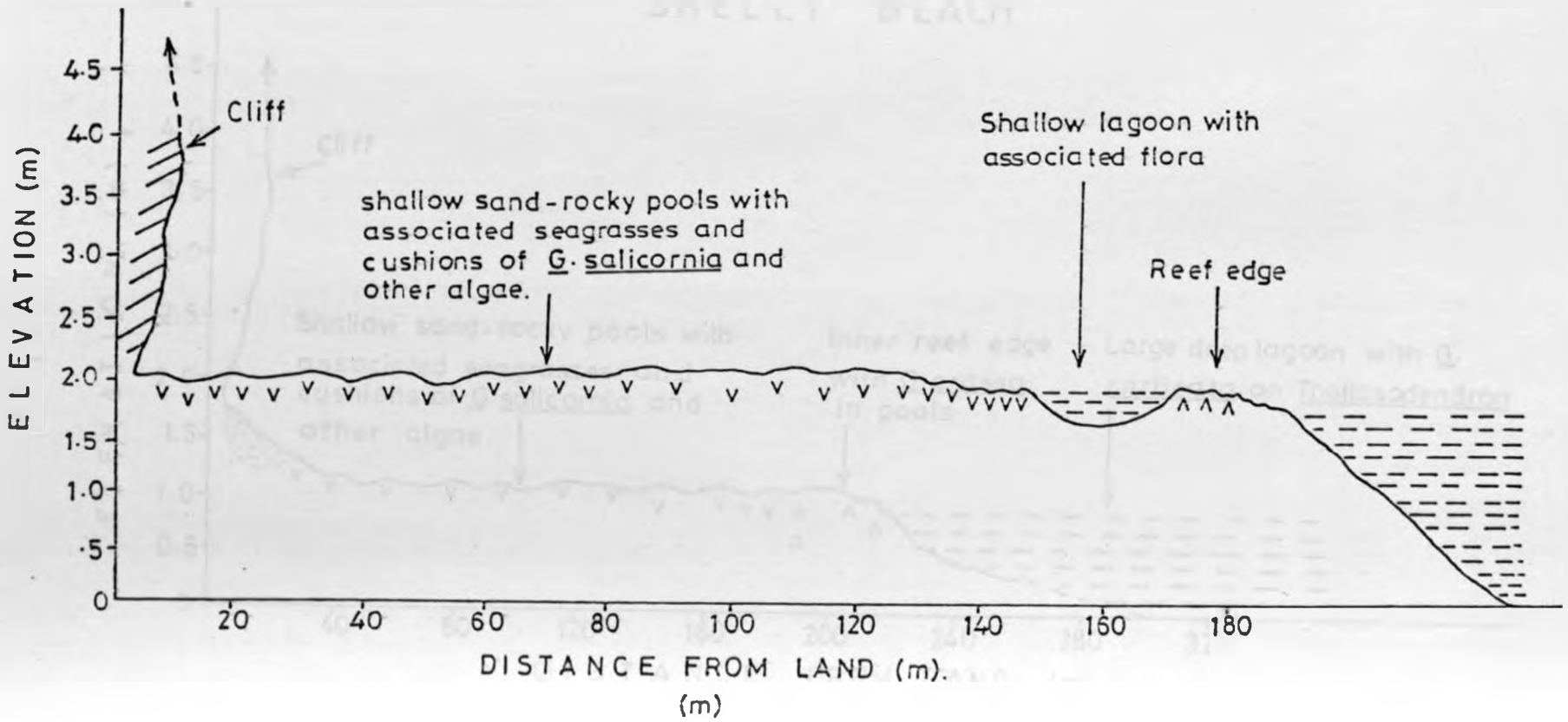
(k)

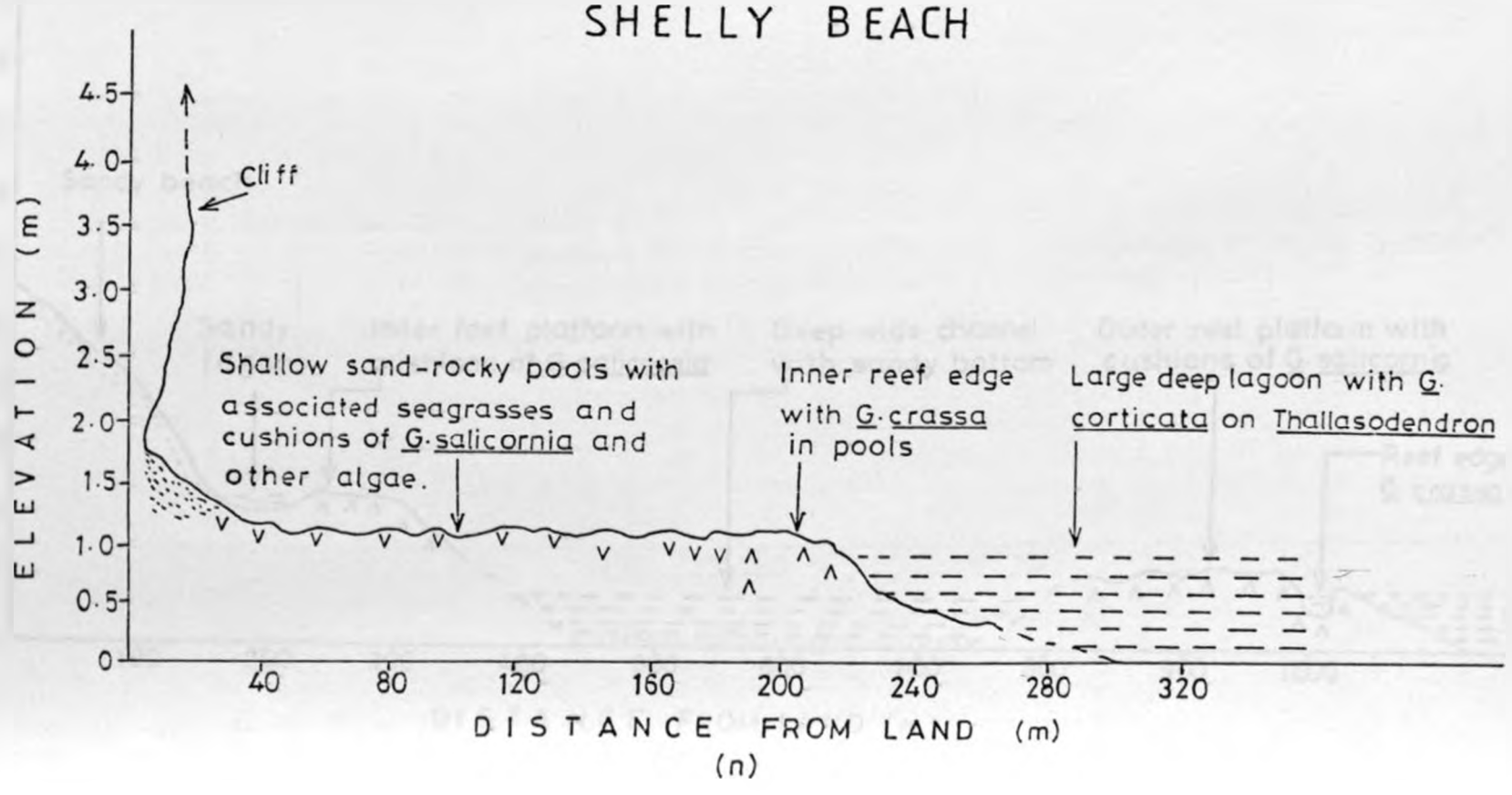
# MOMBASA HOSPITAL

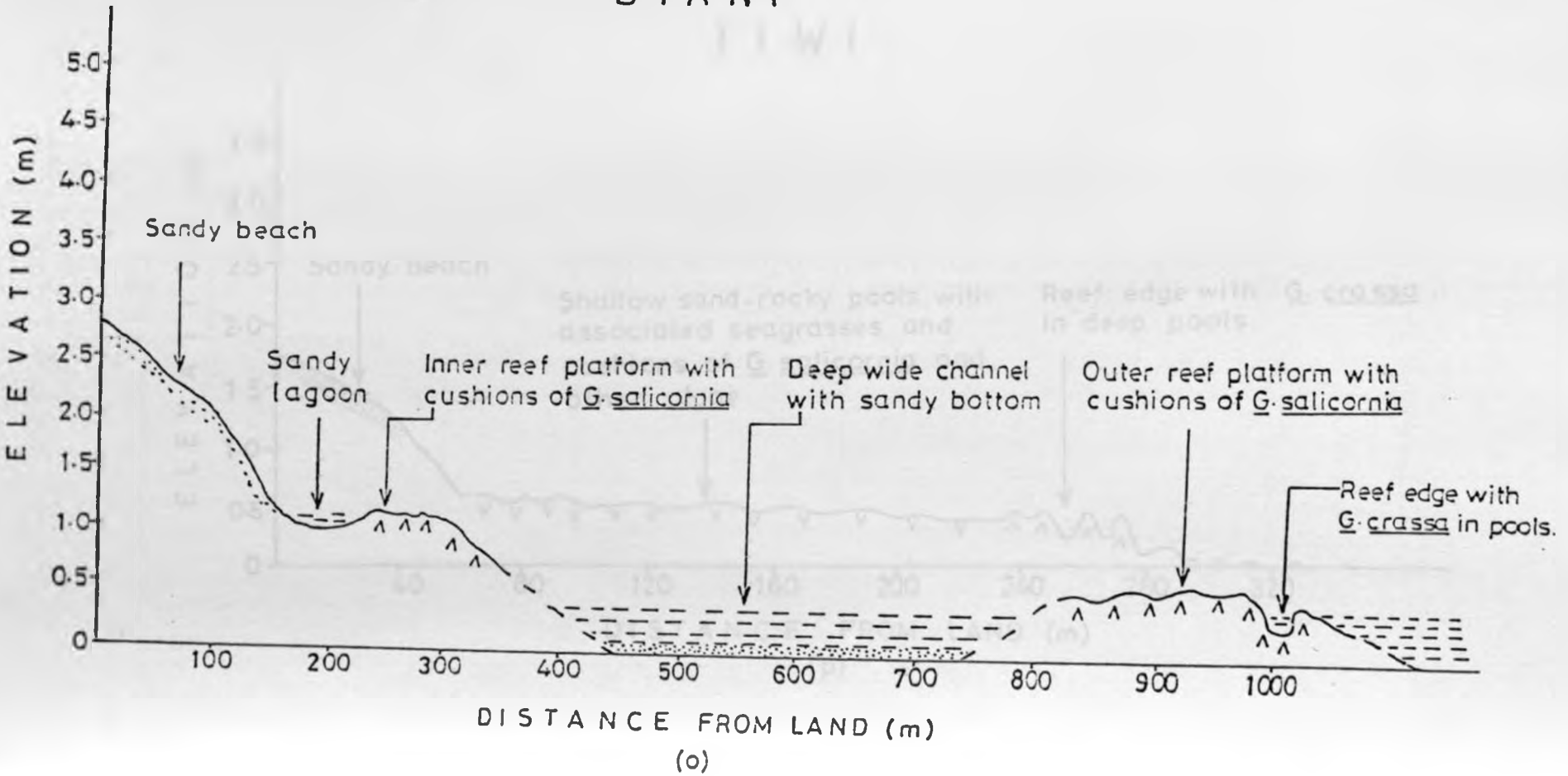



(1)

# LIKONI

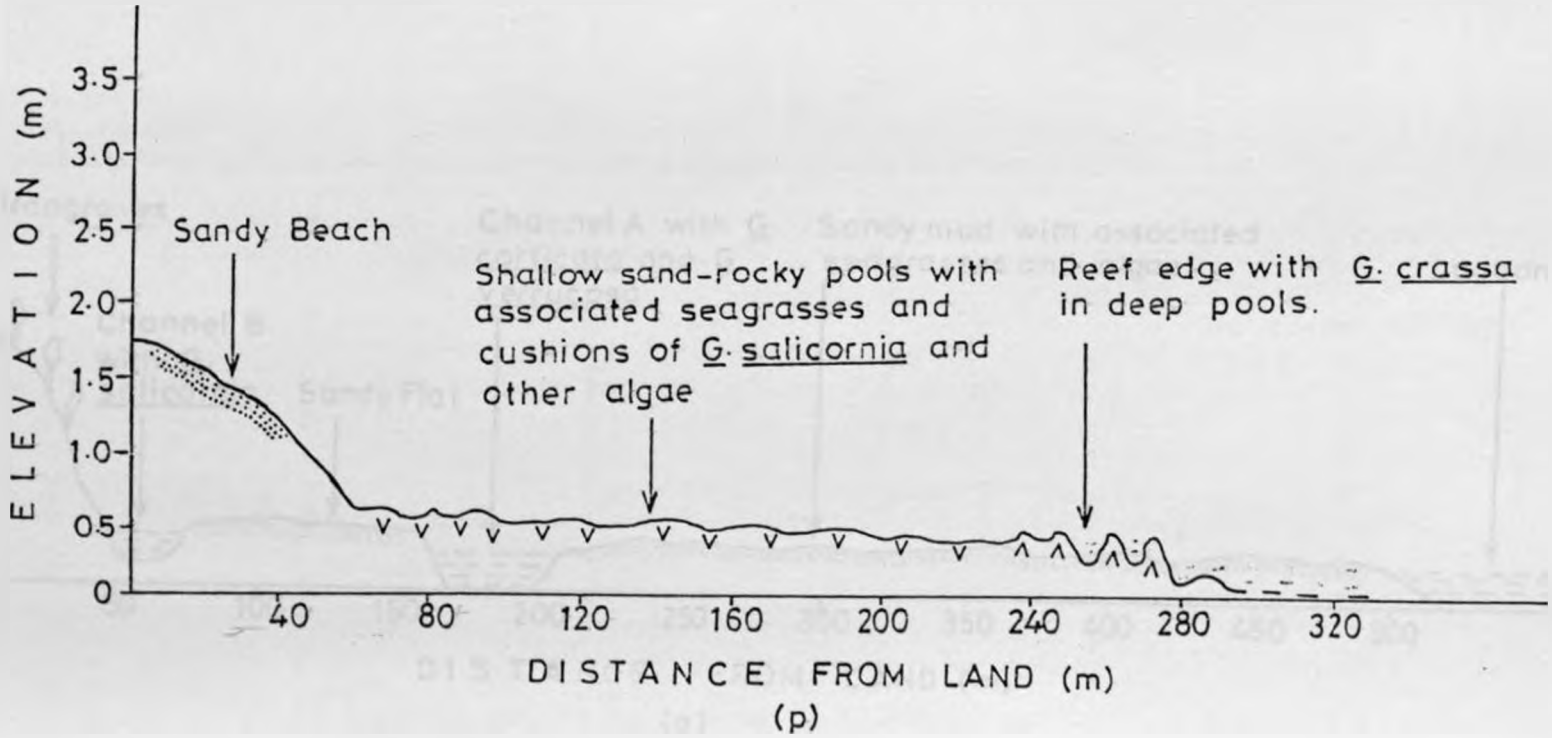

Cliff
shallow sand-rocky pools with associated seagrasses and cushions of G. salicornia and other algae.
Shallow lagoon with associated flora
Reef edge
ELEVATION (m)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0
20
40
60
80
100
120
140
160
180
DISTANCE FROM LAND (m).
(m)


SHELLY BEACH
ELEVATION (m)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Cliff
Shallow sand-rocky pools with associated seagrasses and cushions of G. salicornia and other algae.
Inner reef edge with G. crassa in pools
Large deep lagoon with G. corticata on Thallasodendron
40
80
120
160
200
240
280
320
DISTANCE FROM LAND (m)
(n)

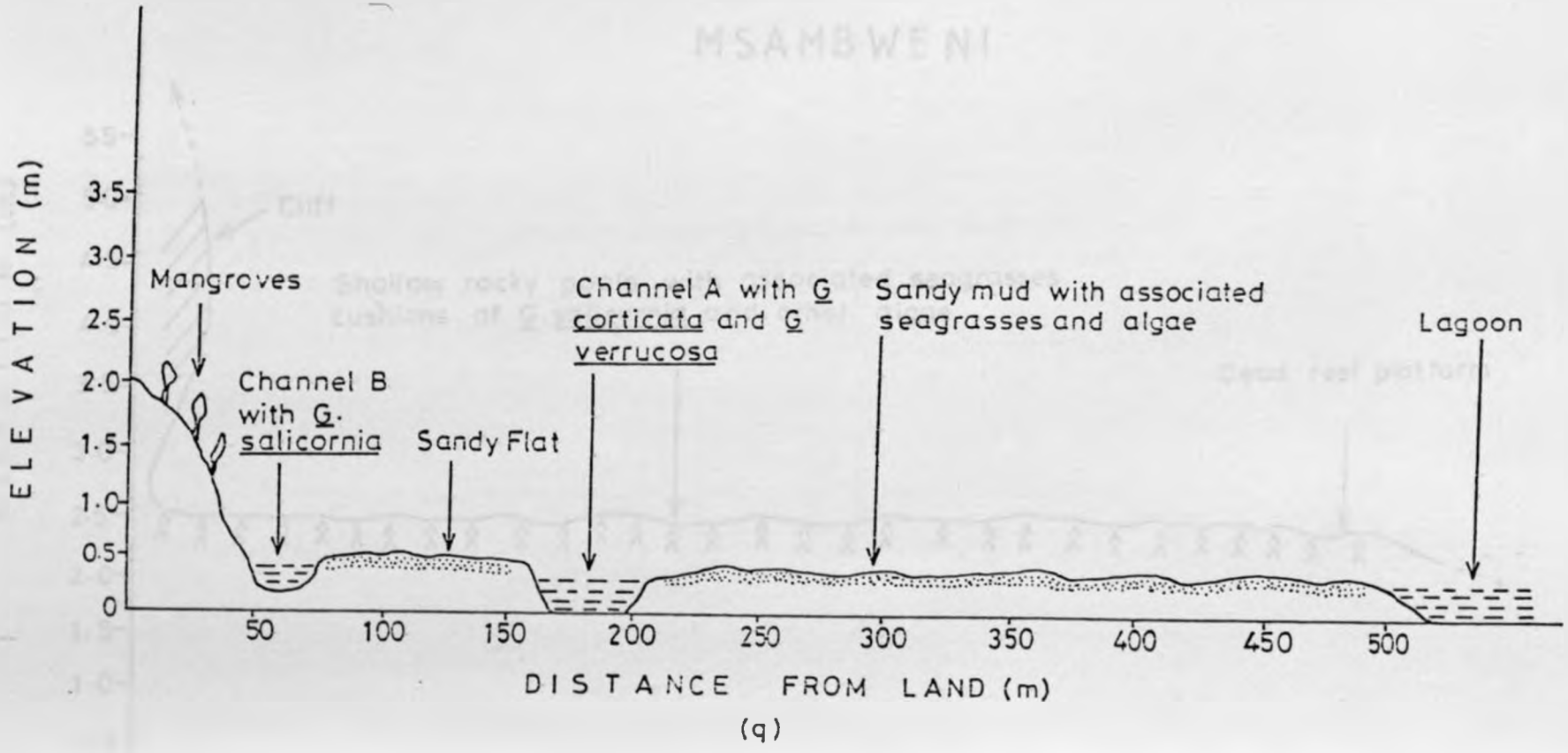

DIANI
ELEVATION (m)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Sandy beach
Sandy lagoon
Inner reef platform with cushions of G. salicornia
Deep wide channel with sandy bottom
Outer reef platform with cushions of G. salicornia
Reef edge with G. crassa in pools.
100
200
300
400
500
600
700
800
900
1000
DISTANCE FROM LAND (m)

(o)

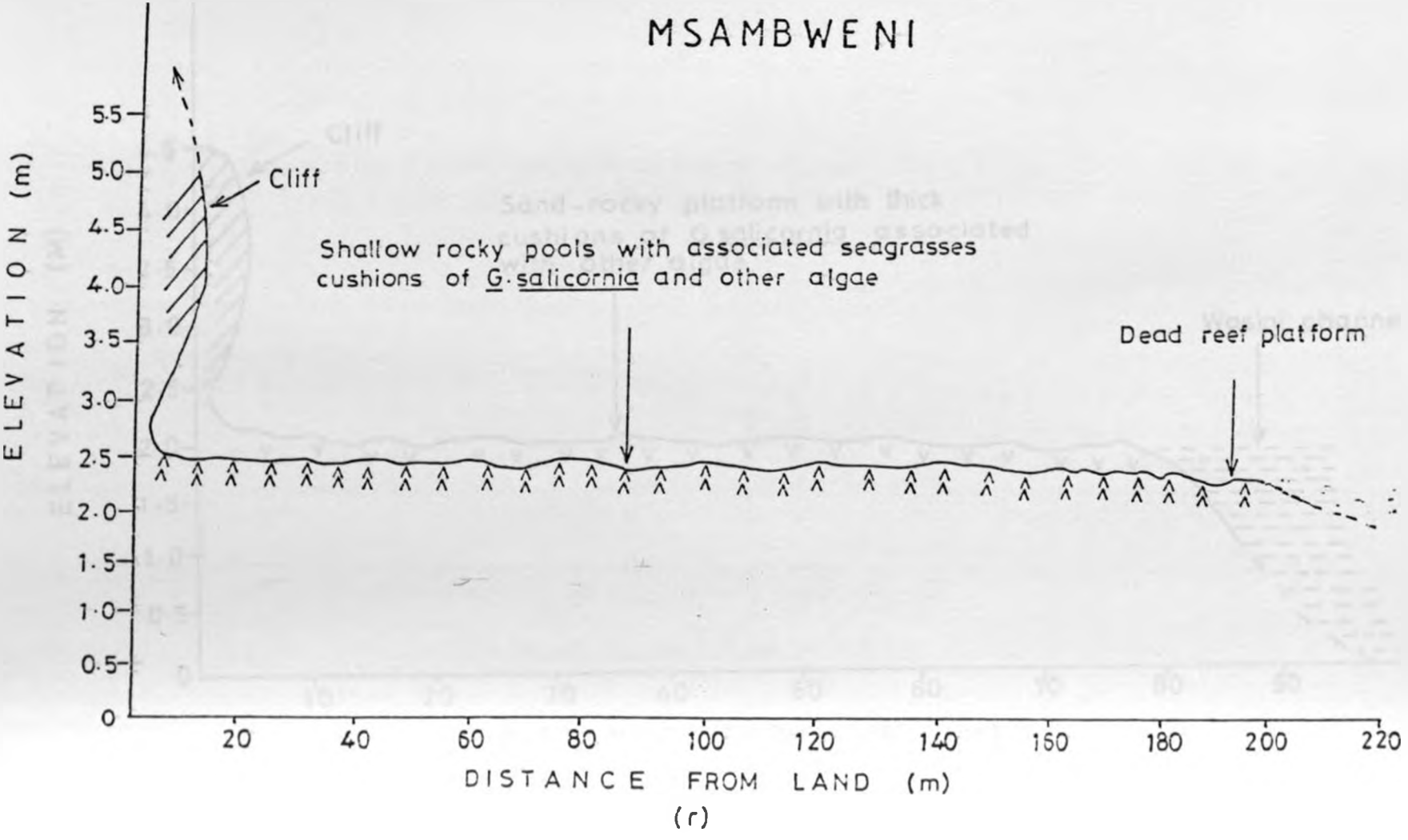
# T I W I


ELEVATION (m)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Sandy Beach
Shallow sand-rocky pools with associated seagrasses and cushions of G. salicornia and other algae
Reef edge with G. crassa in deep pools.
40
80
120
160
200
240
280
320
DISTANCE FROM LAND (m)
(p)

# GAZI BAY

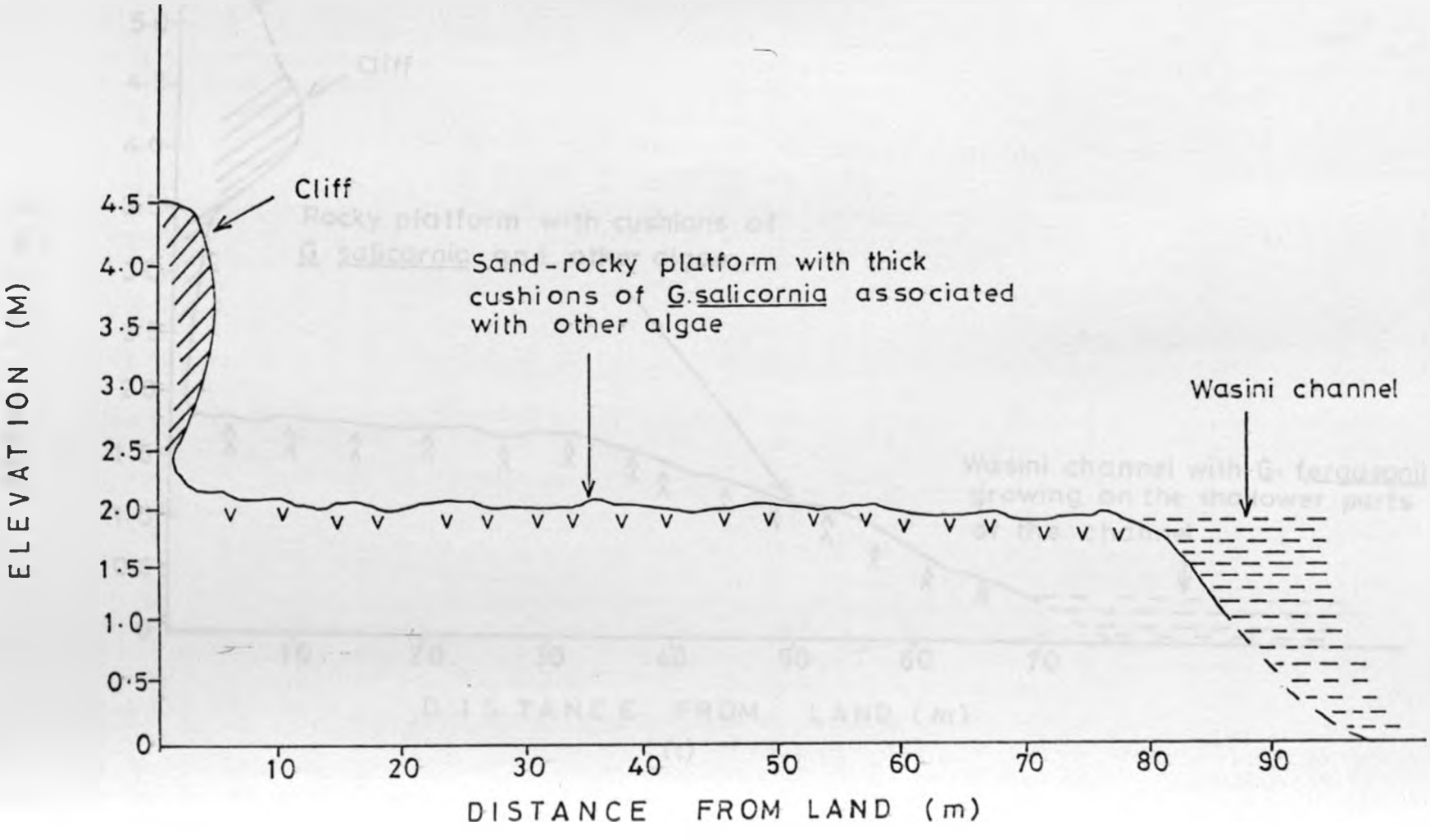

ELEVATION (m)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Mangroves
Channel B with G. salicornia
Sandy Flat
Channel A with G corticata and G verrucosa
Sandy mud with associated seagrasses and algae
Lagoon
50
100
150
200
250
300
350
400
450
500
DISTANCE FROM LAND (m)

(q)

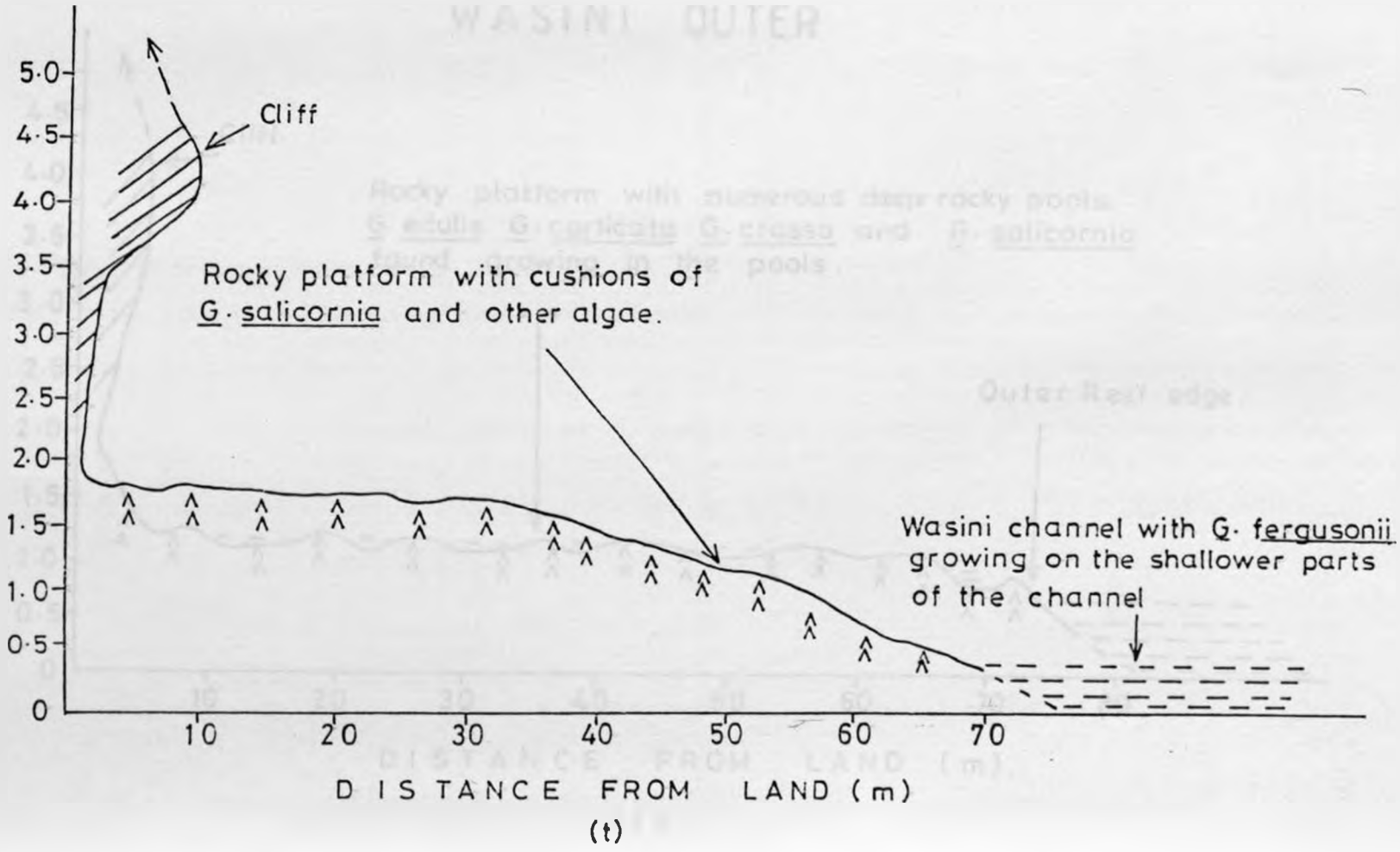

MSAMBWENI
ELEVATION (m)
5·5
5·0
4·5
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
Cliff
Shallow rocky pools with associated seagrasses
cushions of G. salicornia and other algae
Dead reef platform
20
40
60
80
100
120
140
160
180
200
220
DISTANCE FROM LAND (m)

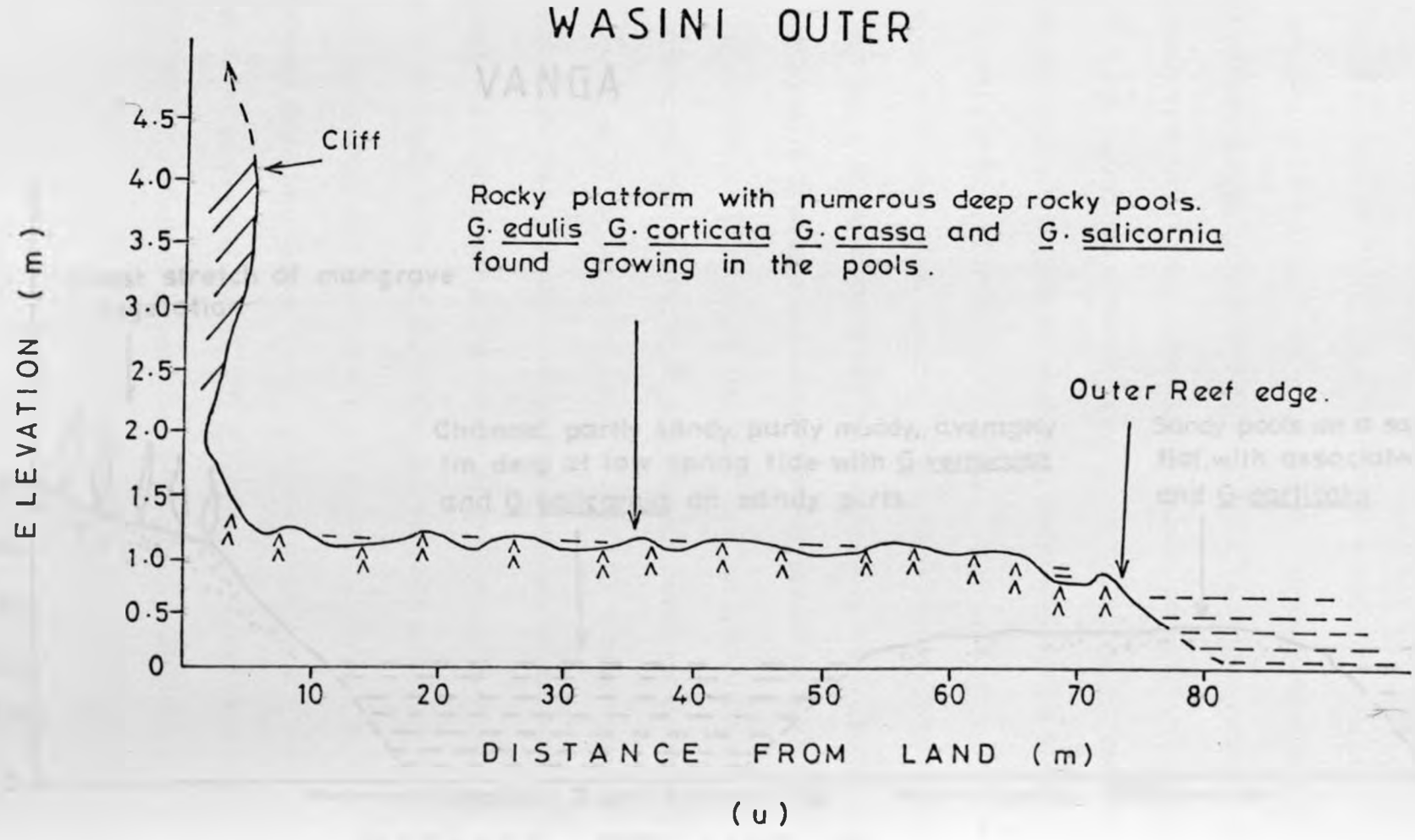
(r)

SHIMONI MAINLAND.

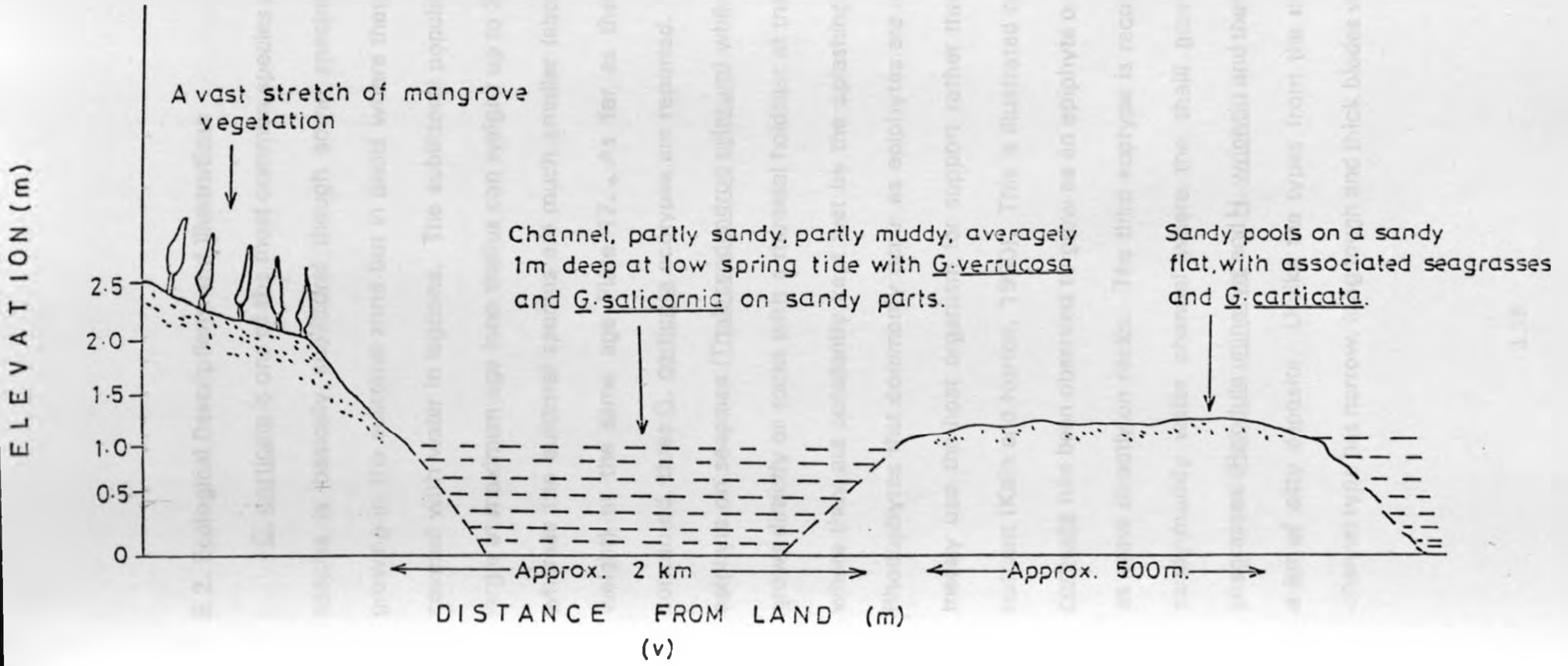



(s)


WASINI INNER
ELEVATION (m)
5·0
4·5
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
Cliff
Rocky platform with cushions of G. salicornia and other algae.
Wasini channel with G. fergusonii growing on the shallower parts of the channel
10
20
30
40
50
60
70
DISTANCE FROM LAND (m)
(t)


WASINI OUTER
Cliff
Rocky platform with numerous deep rocky pools.
G. edulis G. corticata G. crassa and G. salicornia
found growing in the pools.
Outer Reef edge.
ELEVATION (m)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
70
80
DISTANCE FROM LAND (m)

(u)


VANGA
A vast stretch of mangrove vegetation
Channel, partly sandy, partly muddy, averagely 1m deep at low spring tide with G. verrucosa and G. salicornia on sandy parts.
Sandy pools on a sandy flat, with associated seagrasses and G. carticata.
ELEVATION (m)
2.5
2.0
1.5
1.0
0.5
0
Approx. 2 km
Approx. 500m.
DISTANCE FROM LAND (m)

(v)

## 5.2. Ecological Descriptions and Illustrations

G. corticata is one of the most common species of Gracilaria. The species is basically sublittoral though some specimens were found growing in the eulittoral zone but in pools where they were constantly covered with water in lagoons. The sublittoral populations grow much larger at maximum age (one thallus can weight up to 390g wet weight) whereas the eulittoral species are much smaller (about 19 - 25g wet weight) at the same age Plate 17. As far as their substrates are concerned, three G. corticata ecotypes are reported. One grows as an epiphyte on seagrass (Thalassodendron ciliatum) while the second type grows directly on rocks with a rhizoidal holdfast at the edge of the reef where they are constantly kept wet by the splashing waves Plate 18. Rhodophytes that commonly occur as epiphytes are non-obligate, they merely use the host organism for support rather than as a source of nutrient (Kain and Norton, 1990). This is illustrated by the fact that G. corticata has been observed to grow as an epiphyte on seagrass as well as grow directly on rocks. The third ecotype is recorded growing in a sandy-muddy water channel where the thalli grow entangled with seagrasses (Halodule uninervis and H. wrightii) and they are covered with a lot of silty deposits. Unlike the types from the other habitats, the channel type has narrow, long tough and thick blades with no projections

on their margins. The narrow blade with no projection is likely to aid in the reduction of hydrodynamic drag (Koehl, 1986) whereas the thickness of the blade provides structural strength to withstand the force of wave action along the channel.

From the distribution study G. crassa can be reported as one of the species also commonly found along the coast, though thalli were observed in isolation in most stations. It was only at one station (Mombasa Hospital) that a population was observed where most of the follow up studies were carried out. The species is mainly eulittoral, growing in rocky pools at the reef edge. Most of the pools are deep ranging from 20cm to 1m deep and they occur in hidden crevices. The occurrence of the species is such that it is never exposed to desiccation at any time. The species attaches itself to the rocks with its rhizoidal holdfast. Plate 19.

G. edulis is one of the species that is not of common occurrence along the coast. The thalli encountered were in isolation and the species is basically eulittoral. It grows in shallow sandy ponds in association with seagrass (Thalassia hemprichii) and it also grows in rocky pools.

G. fergusonii is one of the rare species. It is strictly sublittoral growing in submerged rocky areas in association with other sublittoral species such as Sargassum. It attaches itself onto the rocks by a discoid holdfast.

G. millardetii is another rare species. The species is eulittoral growing in shallow rocky pools with sandy bottoms. The pools are up to 15cm deep. At the only station where it was found it was observed that it flourished more in the pools which had lowered salinities due to seepage of fresh water from underground. The pools without seepages had lesser densities of the plant. It was also observed that during its peak period of cover there is a lot of other green algae, especially Ulva fasciata. and Enteromorpha kylinii growing entangled with the species. This is an indication of the area having high nutrient levels which is confirmed by the results of nutrient studies in the next chapter. Plate 20.

The most common of all the species studied is G. salicornia. It occurs virtually at all sampling stations and in every type of microhabitat described. The species is basically eulittoral though some small percentage is in the sublittoral. The eulittoral species range from those that are completely exposed to desiccation during low tide to those that

are permanently covered by water in pools. On the basis of their substrates four ecotypes are recorded. One type is observed growing in shallow pools of up to 10cm deep with sandy bottoms though they are mainly attached to rocky patches of the pools. Their thalli are thin, about 1mm in diameter and they grow up to a length of 10cm high. The other type grows in pools that are slightly deeper, up to 50cm deep and are shaded either by cliffs or by other larger species of algae. The thalli of this type are bigger in diameter (averagely 3mm) and are of robust growth. This type is also found on intertidal flats that have rock-muddy substrates which are kept wet by shallow pools of water. In such microhabitats they grow in bundles as big as up to 20cm wide.

The third ecotype of G. salicornia grows directly on exposed rocky surfaces thus forming a continuous thick olive green cushion on the rocks. There is a thin layer of sand on these rocks. The cushions range from 3-5cm high and can be as wide as the rocky surface extends. This ecotype that forms cushions was observed to form big bundles of cushions loosely attached to the rocky surface in areas that had wet sandy-muddy surfaces. The final type is the sublittoral type which has been reported in a sandy-muddy-channel. This type grows much larger than any other type, up to 25cm long and they grow on dead pieces of

mangrove roots or branches or on pieces of coral rocks. It sometimes exhibits a segmented appearance on the thalli due to the constrictions that occur on them. The difference in size between the intertidal and the sublittoral populations conforms to a report by Edwards (1977) that all the intertidal red algae tested in his investigation grew better when permanently submerged. Taylor and Hay (1984) also reported that aggregation of seaweeds into tufts decreases their productivity per gramme organic weight due to crowding of thalli, though it increases resistance to desiccation. They also showed that distribution of the tuft growth form is correlated with the intensity of desiccation stress. On the other hand the non-tuft growth form allowed higher productivity. Plates 21 and 22.

G. verrucosa is yet another rare species that was not very common. It was recorded in sandy channels where it was directly attached to the sand. Its occurrence was particularly observed during the cool months of the year, that is July-August. Of the species studied this is the only one that was observed to thrive mainly on purely soft unstable substratum composed of sandy-silt mixtures. The population was stabilised by the basal parts being buried in the soft sandy sediments. McLachlan and Bird (1986) reported that one of the ways through which

Gracilaria growing on soft substratum stabilise themselves is by partially being buried in the soft sediments. In his study on the same species Jones (1959) suggested that in the pools, exposure to full light has a deleterious effect on the plant. This tallies with the present study whereby it was observed that the populations in pools grew much shorter than in deeper waters. It was noted that G verrucosa was only recorded at stations that have fresh water input through rivers or underground seepage.

Gracilaria sp. is one of the intertidal species that was not very common. It was observed growing in the inner edges of the intertidal pools on the reef platforms, attached to the rocky substrates by holdfasts.

Plate 17. Habit of the largest epiphytic *G. corticata*

Plate 18. Ecological habitat for the rocky type G. corticata.

Plate 19. Ecological habitat for

G. crassa


Plate 20. Ecological habitat for

G. millardetii

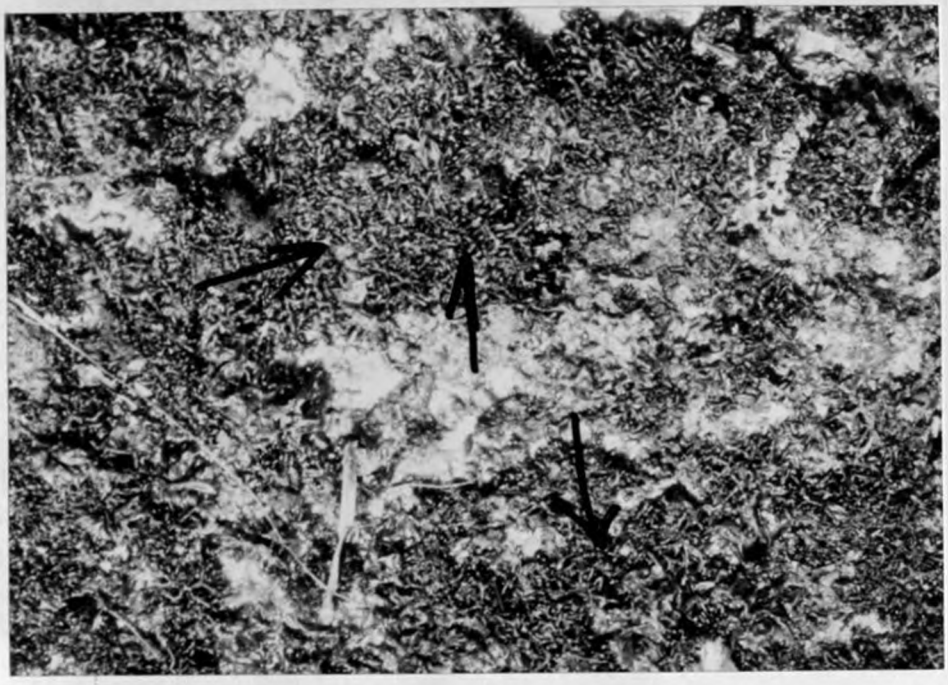


Plate 21. Ecological habitat for G. salicornia (intertidal cushion type).

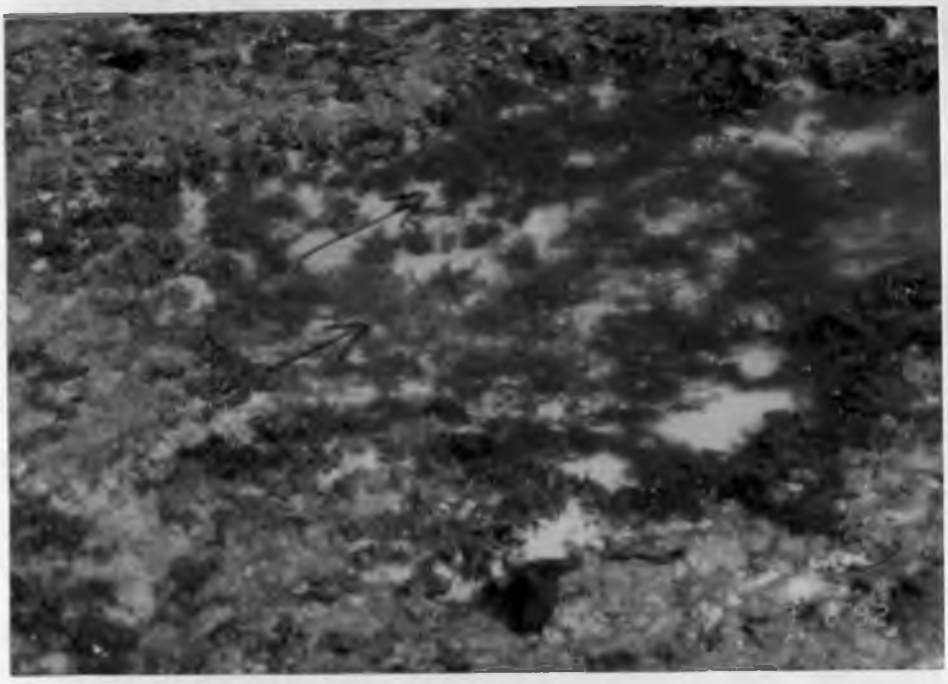


Plate 22. Ecological habitat for G. salicornia (intertidal pool type).

Plate 23. Ecological habitat for G. salicornia (intertidal bundle forming type).

## 5.3. The Distribution of Gracilaria

The distribution study of the Gracilaria species of the Kenya coast show that G. salicornia is the most commonly found species since it was observed at all sampling stations. G. corticata is the next common followed by G. crassa. The rest of the species i.e. G. fergusonii, G. verrucosa, G. millardetii, G. edulis and Gracilaria sp. could be considered as rare species as far as their distribution is concerned. Table 1. Of the different ecotypes of G. corticata the epiphytic type was the most common while for G. salicornia the cushion forming type was quite abundant compared to the other types. The north-south distribution of the species has not shown any specific trend. However, stations with rocky platforms had a wider variety of species than the sandy or mangrove beaches. Though no quantitative studies were carried out on standing crop of the different species, it is evident from field studies that G. salicornia is the most abundant followed by G. corticata in terms of biomass. It is therefore, a general feature throughout the study that G. salicornia tends to grow best and subsequently is the dominant species in conditions where other species are poorly represented.

From this study it is evident that most of the Gracilaria species grow on rough substrate. Rough substrata enhance the settlement,

anchorage and subsequent survival of the spores (Harlin and Lindbergh, 1977; Ogata, 1953). They are held up in the depressions of the microtopography. When the tide recedes spores lodged in depressions are likely to be in a more humid microclimate than those on open rocks, and they may also receive some protection from grazers (Kain and Norton, 1990). Observations made by McLachlan and Bird (1986) showed that when attached plants of Gracilaria occur on extensive solid substratum they are usually accompanied by sand or other poorly consolidated sediments. This was a very common feature in this study whereby there was always some sandy sediments between the plants and the rocky substrate.

The survey has revealed that there are no large expanses of Gracilaria beds at any one of the stations sampled that could be compared to those of the temperate regions. Most of the populations are found in discrete patches while some occur as isolated thalli. The only Gracilaria population that could be referred to as a large population in this study is that of G. corticata at McKenzie Point station which covers an area of about 100m$^2$. Even within this area there are patches without any growth of the species. This observation could be explained by the suggestion given by McLachlan and Bird (1986) that maximum growth

and productivity for the genus is limited by the high temperatures experienced in the tropics. They further suggested that little fluctuation in temperatures in tropical areas subject some species to exist at or near supra-optimal conditions throughout the year thus reducing their productivity, resulting in less standing stock.

TABLE 2. THE DISTRIBUTION OF GRACILARIA SPECIES ALONG THE KENYA COAST

| SPECIES | STATIONS | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| G. corticata J. Agardh | x | | x | x | x | x | | x | x | | x | | x | | x | | | x | | | x | | x |
| G. crassa Harvey | | | x | x | | | | x | | x | x | | x | | x | x | x | | | | x | | |
| G. edulis (J.Ag.) Siliva | | | | | | | | x | | | x | | | | | | | | | | x | | |
| G. fergusonii J. Agardh | | | | | | | | | | x | | | | | | | | | | | | x | |
| G. millardetii J. Agardh | | | | | | | | | | | | x | | | | | | | | | | | |
| G. salicornia (J. Ag.) Dawson | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| G. verrucosa (Huds.) Papenfuss | | | | x | | | | | | | | | | | | | | x | | | | | x |
| Gracilaria sp. | | | x | | | | | | | | x | | x | | | | | | | | | | |
| Total No. of spp. | 2 | 1 | 4 | 4 | 2 | 2 | 4 | 4 | 2 | 3 | 5 | 2 | 4 | 1 | 3 | 2 | 2 | 3 | 1 | 1 | 4 | 2 | 3 |
| Substrate | S | S | RR | RR | S | S | S | RR | R | RR | RR | RR | RR | R | RR | S | S | S | R | R | RR | R | S |

Key
RR: Rocky platform with reef edge
R: Rocky platform without reef edge
S: Sandy beaches

* **Key To Stations Sampled**

1. Lamu (Manda Island)
2. Ngomeni
3. Mambrui
4. Malindi
5. Watamu
6. Kilifi
7. Vipingo
8. Kanamai
9. Reef Hotel
10. Nyali Beach
11. McKenzie Point
12. Fort Jesus
13. Mombasa Hospital
14. Likoni
15. Shelly Beach
16. Tiwi
17. Diani
18. Gazi
19. Msambweni
20 Shimoni Mainland
21. Wasini (Outer)
22. Wasini (Inner)
23. Vanga

## 5.4. Zonation of Gracilaria

An attempt has been made to study the zonation pattern of Gracilaria. The universal shore zonation schemes adapted for the study (Fig.19) are that of Hartnoll (1976) and Lewis (1964). According to this scheme of zonation the littoral region is divided into three major sub zones. From the shores downwards to the sea, is the first zone, the littoral fringe. This zone gets partly covered by water only during the extreme high water spring (EHWS) while most of the time it only receives splashes of water as a result of the waves breaking on the shores. The following zone is the upper eulittoral zone which lies above the mean tide waters (MTW). This zone gets exposed every day for long hours during low tide periods. The third zone, lower eulittoral zone, gets exposed only during the mean low water spring (MLWS) and does not get exposed during neap tides. From the littoral region the shore then continues into the sublittoral region which is permanently covered with water.

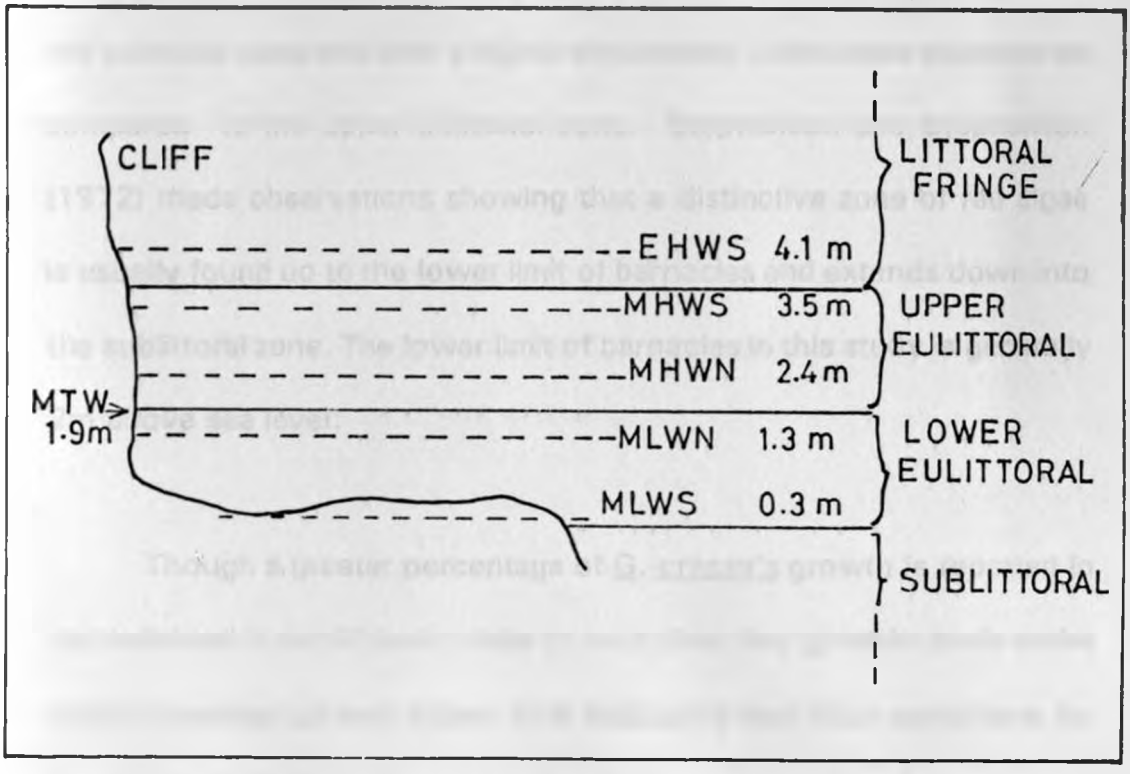



Fig.19. The universal shore terminology adapted for this study.

Of the eight species reported in this study it can be concluded that G. millardetii. sp., G. edulis and Gracilaria sp. are strictly eulittoral whereas G. verrucosa is the only species that is strictly sublittoral. The rest of the species partly grow in the eulittoral zone and partly in the sublittoral. Figure 20 gives a summary of this phenomenon. From this study it comes to light that a larger proportion of the Gracilarias grow in the eulittoral zone and with a higher occurrence in the lower eulittoral as compared to the upper eulittoral zone. Stephenson and Stephenson (1972) made observations showing that a distinctive zone of red algae is usually found up to the lower limit of barnacles and extends down into the sublittoral zone. The lower limit of barnacles in this study is generally 2m above sea level.

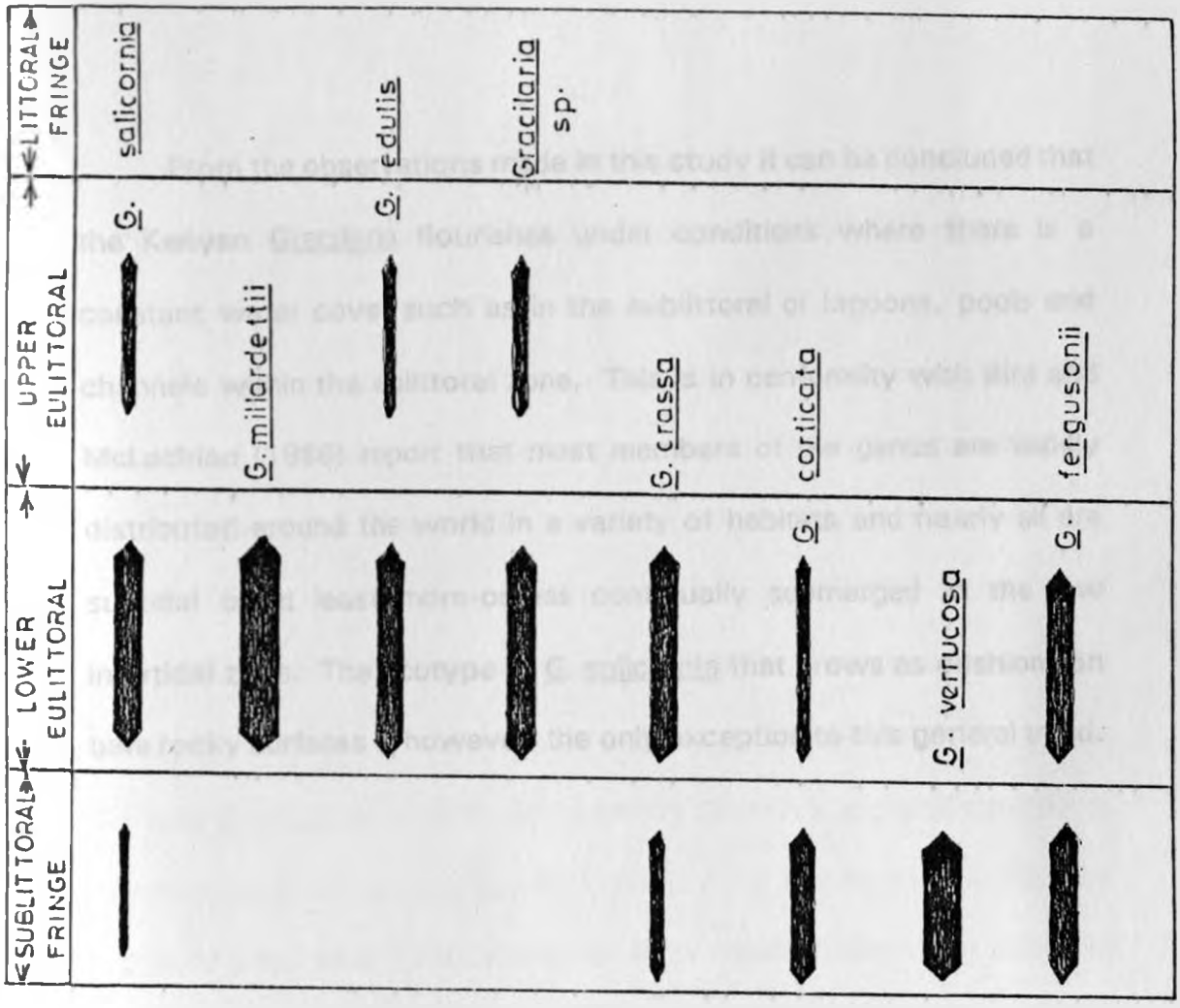
Though a greater percentage of G. crassa's growth is reported in the eulittoral it would be in order to note that they grow in pools under hidden crevices at reef edges thus indicating that their conditions for growth are such that they are never exposed to direct insolation even at lowest tide. The temperatures in these pools are generally cooler than in the surrounding eulittoral zone pools. The fact that this species grows at the edges of the reef, where wave action on the plants is rough, explains the tough fleshy smooth cushion nature of the species which

would otherwise be broken off easily. Their concentration on the lower eulitteral zone is an indication that they do not get exposed for very long hours during low tides.

Populations of G. corticata that are found in the eulittoral grow near the edge of the reef in pools that never dry up. The ecotype that grows directly on the rocks at the reef edge also get constant splashes of water as the waves break on the reef edge. The populations that grow higher up in the lower eulittoral zone do so in lagoons where they are permanently covered with water. Since they grow as epiphytes on the lower parts of their host, Thalassodondron ciliatum. they are quite sheltered from the direct effect of insolation.

The rest of the Gracilaria species i.e. G. salicornia, G. millardetii, G. edulis and Gracilaria sp. grow in pools in the eulittoral zone where they are quite exposed to direct insolation during low tides. However, it should be noted that the sublittoral populations of G. salicornia have a more robust growth compared to the eulittoral populations. This could possibly be due to the shelter provided by the water column coupled by availability of nutrients for 24 hours daily. Results obtained from nutrient determinations revealed that some of the eulittoral pools where these

species grow lack nitrates during low tides as evidenced in Chapter 6. This is not the case in the sublittoral conditions. Furthermore a combination of stress from high temperatures during low tides and stress from desiccation is one of the likely causes of stunted growth of G. salicornia in the eulittoral zone. Work done by Hodgson (1984) showed similar observations.




KEY

| | |
|---|---|
| ⊣ | < 20% |
| ⊣ | 20-40% |
| ⊣ | 40-60% |
| ⊣ | 60-80% |
| ⊣ | Over 80% |

Fig.20. Relative Zonation of the different Gracilaria species showing percentage of occurence at different zones

From the observations made in this study it can be concluded that the Kenyan Gracilaria flourishes under conditions where there is a constant water cover such as in the sublittoral or lagoons, pools and channels within the eulittoral zone. This is in conformity with Bird and McLachlan (1986) report that most members of the genus are widely distributed around the world in a variety of habitats and nearly all are subtidal or at least more-or-less continually submerged in the low intertidal zone. The ecotype of G. salicornia that grows as cushions on bare rocky surfaces is however, the only exception to this general trend.

## CHAPTER 6

## SEASONALITY AND GROWTH STUDIES OF GRACILARIA

Literature has shown that seaweeds exhibit seasonality in their growth behaviour and that different taxa depict different patterns in their seasonality which vary with time and geographical location (Humm, 1951; John and Asare, 1975; Penniman, 1977; Trono and Azanza-Corrales, 1981; Umamaheswara, 1969). This study was carried out with a view to establishing the specific seasonality patterns of the Kenyan Gracilaria species as influenced by the prevailing local conditions. The investigation was spread over a period of 12 months from September 1989 to September 1990, covering all the major seasons that influence the growth of the plants. For the purpose of regular monthly sampling, proximity of the sampling stations and availability of the specimens determined the species studied.

### 6.1 Seasonality of individual species.

G. corticata

Observations for the population at McKenzie station showed maximum cover during the months of September through to February. During November fertile plants were observed and this reached its peak

in December. By January some thalli had already started getting detached and dying off and no fertile plants were observed. Lots of new growth was observed on the old thalli that were left thus giving them a very rough appearance. This condition persisted till the month of April when most plants were dead and floating in water. By May there was not a single thallus left in this locality and this lasted through June. In July new growths started shooting off on seagrasses and this marked the season for regeneration which reached its peak in September and the cycle was then repeated. The population at Gazi exhibited a similar pattern of seasonality though the population was not as dense as the one at McKenzie station. Figure 21.

G. salicornia

The intertidal population at McKenzie showed maximum cover during August through to January after which it started decreasing. During the period of maximum cover it was growing in association with some green algae which formed dense cover over the population. The chlorophytes were Ulva fasciata, U. reticulata, Enteromorpha kylinii and E. clathratus. During the subsequent months up to April the population decreased in number and the few thalli left were bleached and of stunted growth. By May the regeneration had started and this reached its peak

in September. Throughout this period of growth no fertile plants were observed. However, plants were observed in the subtidal population which exhibited the same pattern of seasonality though late by about two months. Fertile specimens were observed just at the period of maximum cover. Figure 22.

G. crassa

The population studied had a period of maximum growth from August through to November after which it started decreasing and getting bleached until March. By April there were signs of new growth. There were shoots coming off the bleached old branches and by August the phase of maximum cover had been reached. A population at Mambrui station had very common fertile plants in March unlike the population studied at Mombasa Hospital which had the poorest growth at this same period of the year. Figure 23.

G. millardetii

The population studied at Fort Jesus showed maximum growth during the months of September to December and thereafter started showing signs of getting bleached. This period of decrease continued till April when the population degenerated completely and not a single

thallus was left. By June regeneration started and this increased till the next phase of maximum growth was reached. The period of maximum growth was also marked by lots of growth of chlorophytes such as Ulva reticulata, U. fasciata, and Enteromorpha kylinii such that these completely shaded the plants. Figure 24.

Gracilaria sp.

An attempt to study seasonality in this species was made though it was not possible to study it regularly on monthly basis as the others due to distance. However, the following observations were made on the few occasions that the station was visited. A visit to the station in November revealed an abundance of the species while a visit in March showed that the population had decreased and most of the thalli were fertile. During a visit in May the population had completely degenerated.

At Mombasa Hospital, a station about 140km south of the first station there was a sudden emergence of a population in August which had not been there the previous month. The cover was about 75%. By the next visit the following month the population started decreasing in cover and by November it had completely degenerated. This was a striking observation because it was during the month of November that

maximum growth of the population at Mambrui was recorded. These differences in the pattern of growth could be due to differences in geographic locations.

G. verrucosa

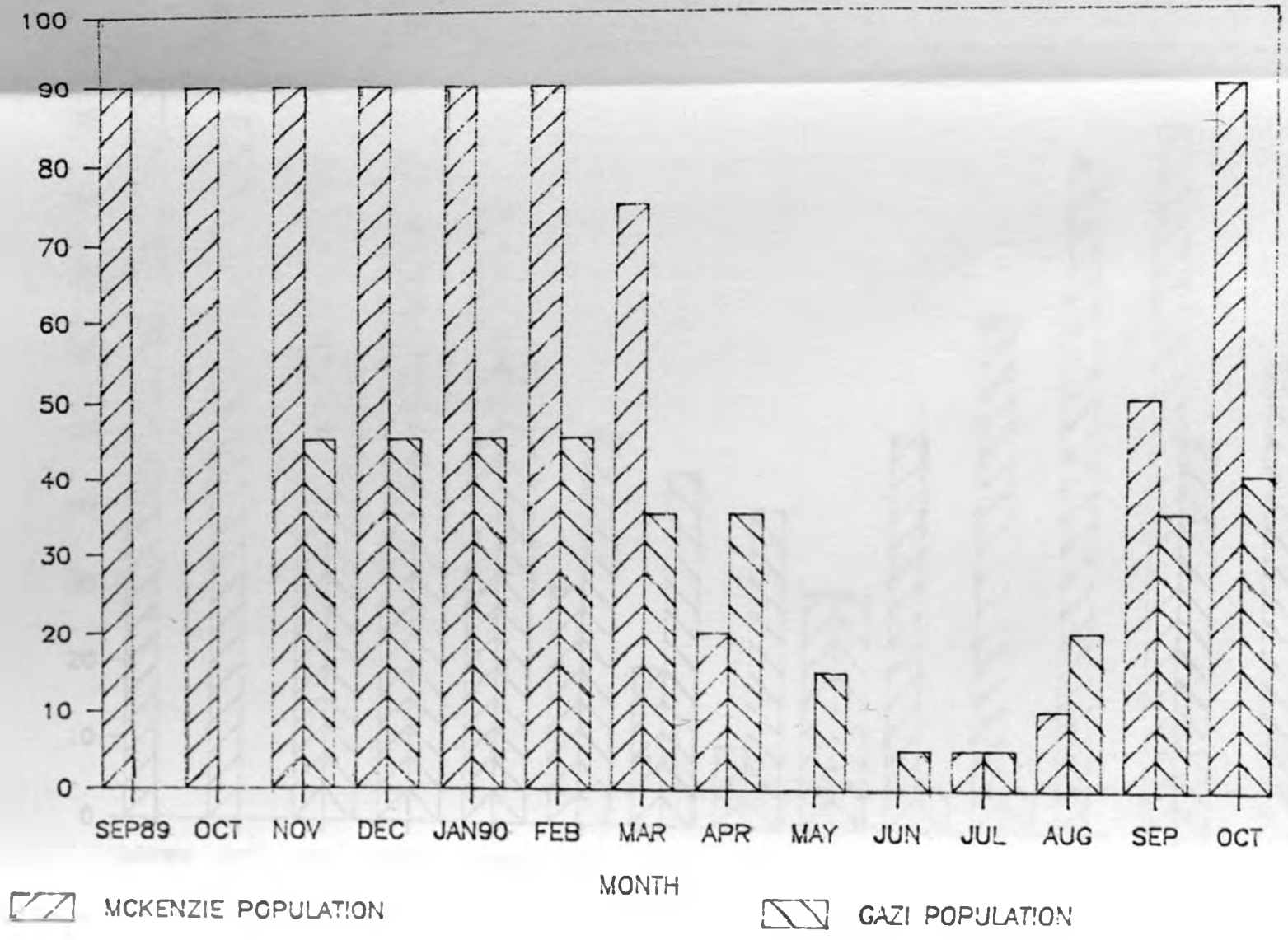
The two populations observed during this study were recorded only during the months of July/August when they occurred in appreciable numbers. During the same period some of the plants were fertile. For the rest of the year not a single thallus was observed. However, towards the end of the project in the month of November, it was noted that there were live thalli buried in sand at Vanga station though it was not possible to quantify the biomass nor follow up their seasonality pattern while buried in sand. A similar phenomenon of thalli being buried in sand for a period of time during their growth cycle has been reported for other Gracilaria species (McLachlan and Bird, 1986; Santelices and Ugarte, 1990; Santelices et al., 1984). The species has also been observed from other parts of the world to have a high growth rate only during a short period of the year, generally about 3 months, and the period varies depending on the latitude (Destombe et al., 1988).

Figure 25 gives a brief summary of the seasonality of the species

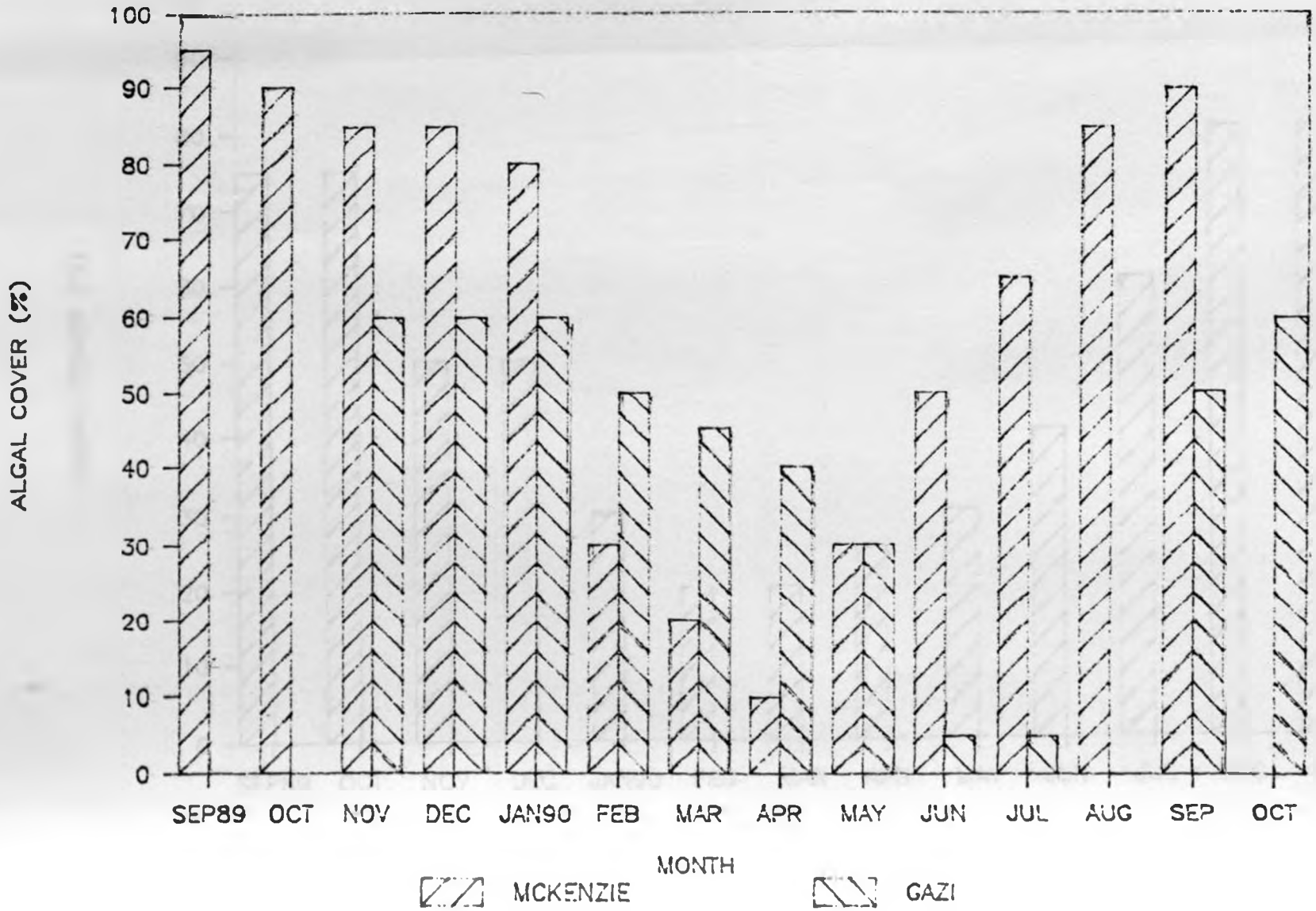
discussed above.

The results obtained in this study show that algal cover for both the G. corticata populations studied was at maximum during the period covering September to March, while that for G. salicornia ranged from August to January. The maximum cover for G. millardetii was September to December while that for G. crassa was August through to November. G. verrucosa had maximal cover in July. It is noteworthy that the seasonal changes of the species varied from complete disappearance of visible thalli in some seasons as in the case of G. corticata, G. millardetii, G. verrucosa and Gracilaria sp., to stunted basal parts as in G. crassa and G. salicornia. It therefore follows that at any given time of the year specimens of G. crassa and G. salicornia are available while those of G. corticata, G. millardetii and G. verrucosa are not available throughout the year. The general trend depicted in this study is that the period for maximal Gracilaria cover is the period covering the end of the South East monsoon and the early part of the North East monsoon seasons. The flora, like any other tropical shore (Untawale et al., 1987) showed a single growing season. This observation is also in accordance with Moorjani's findings (Moorjani, 1977) for the seasonality of the Kenyan seaweeds as a whole.

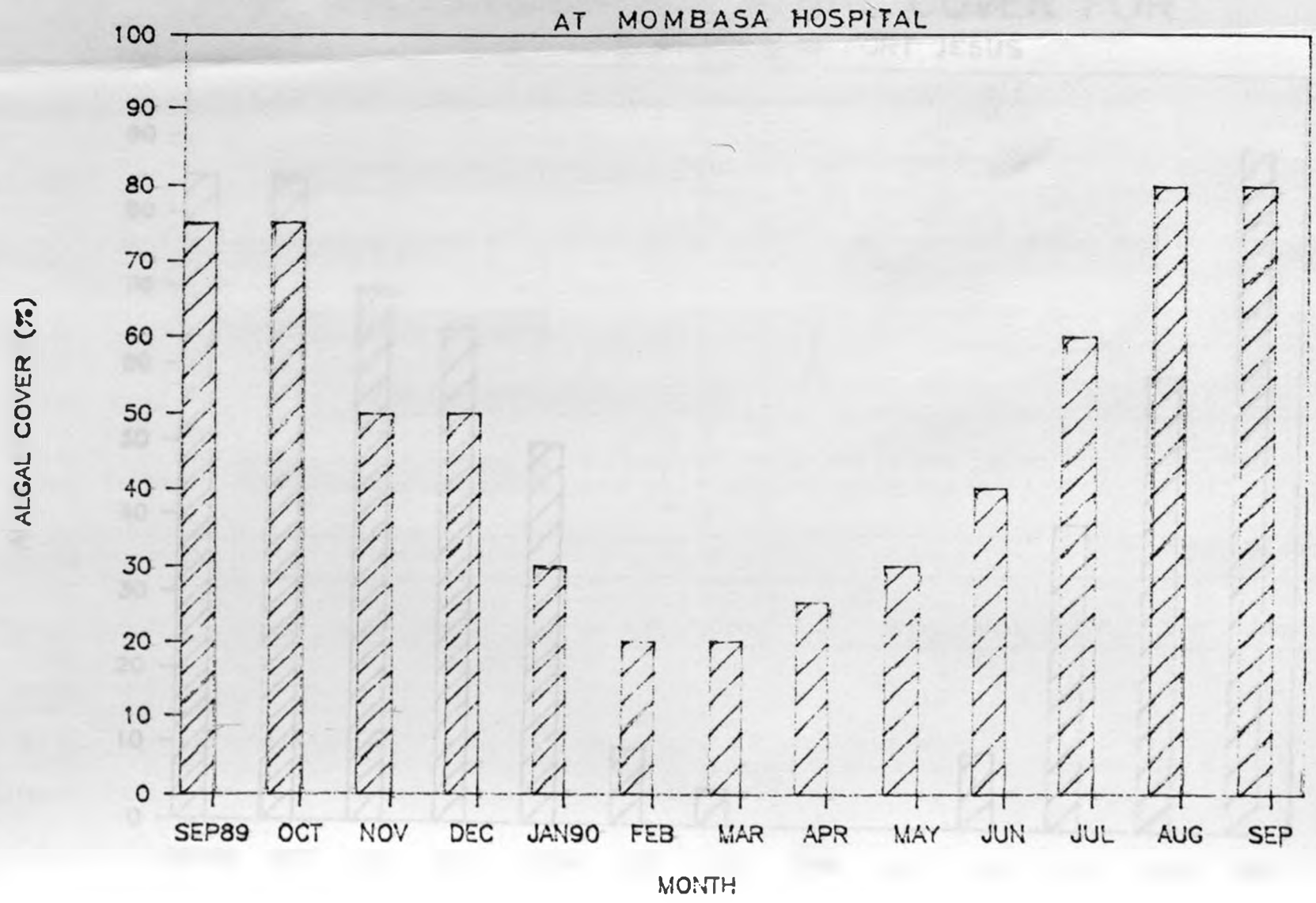
FIG. 21: % ALGAL COVER FOR G.CORTICATA



# FIG. 22: % ALGAL COVER FOR G.SALICORNIA

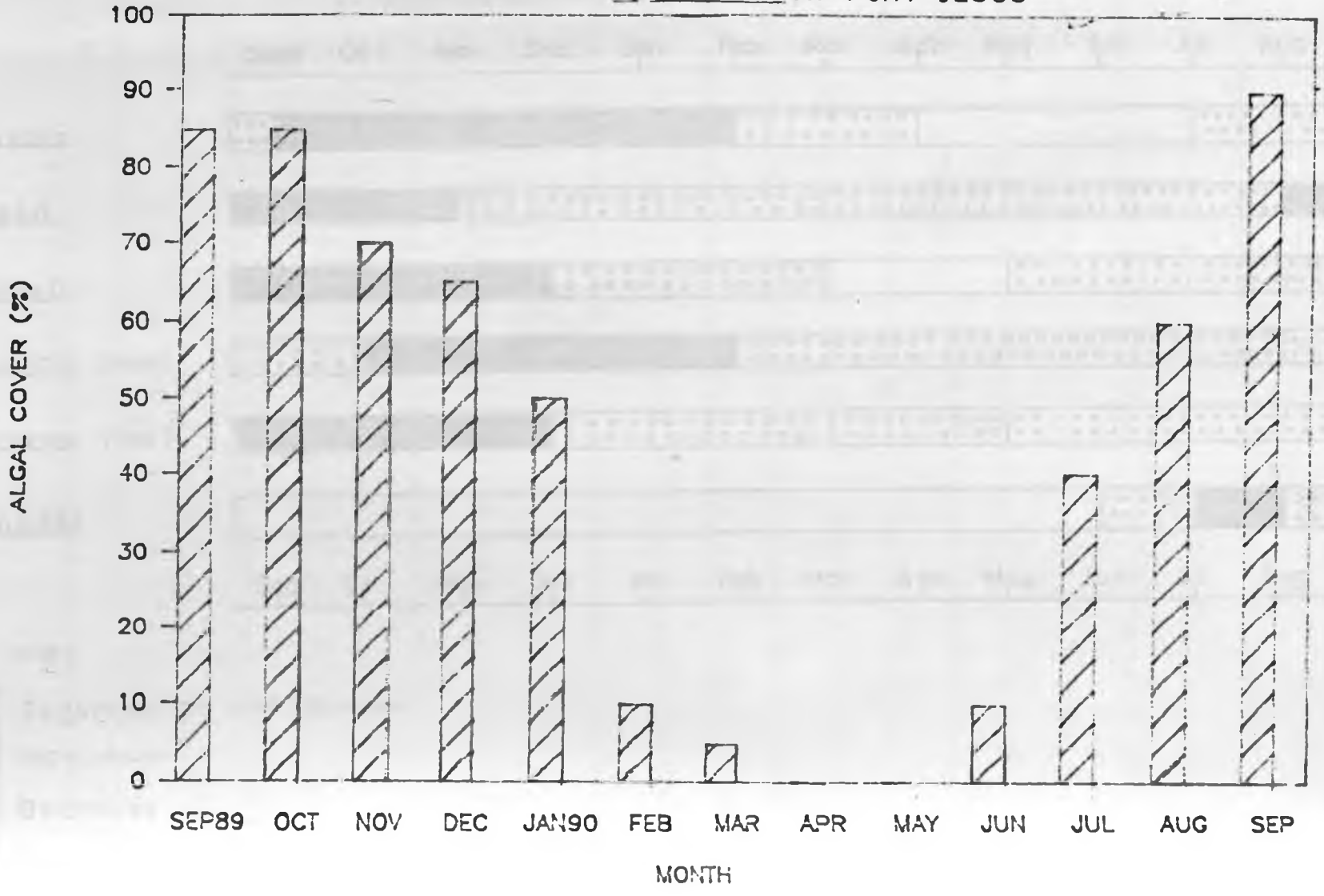


# FIG. 23: % ALGAL COVER FOR G.CRASSA



# FIG. 24: PERCENTAGE ALGAL COVER FOR

G. MILLARDETII AT FORT JESUS



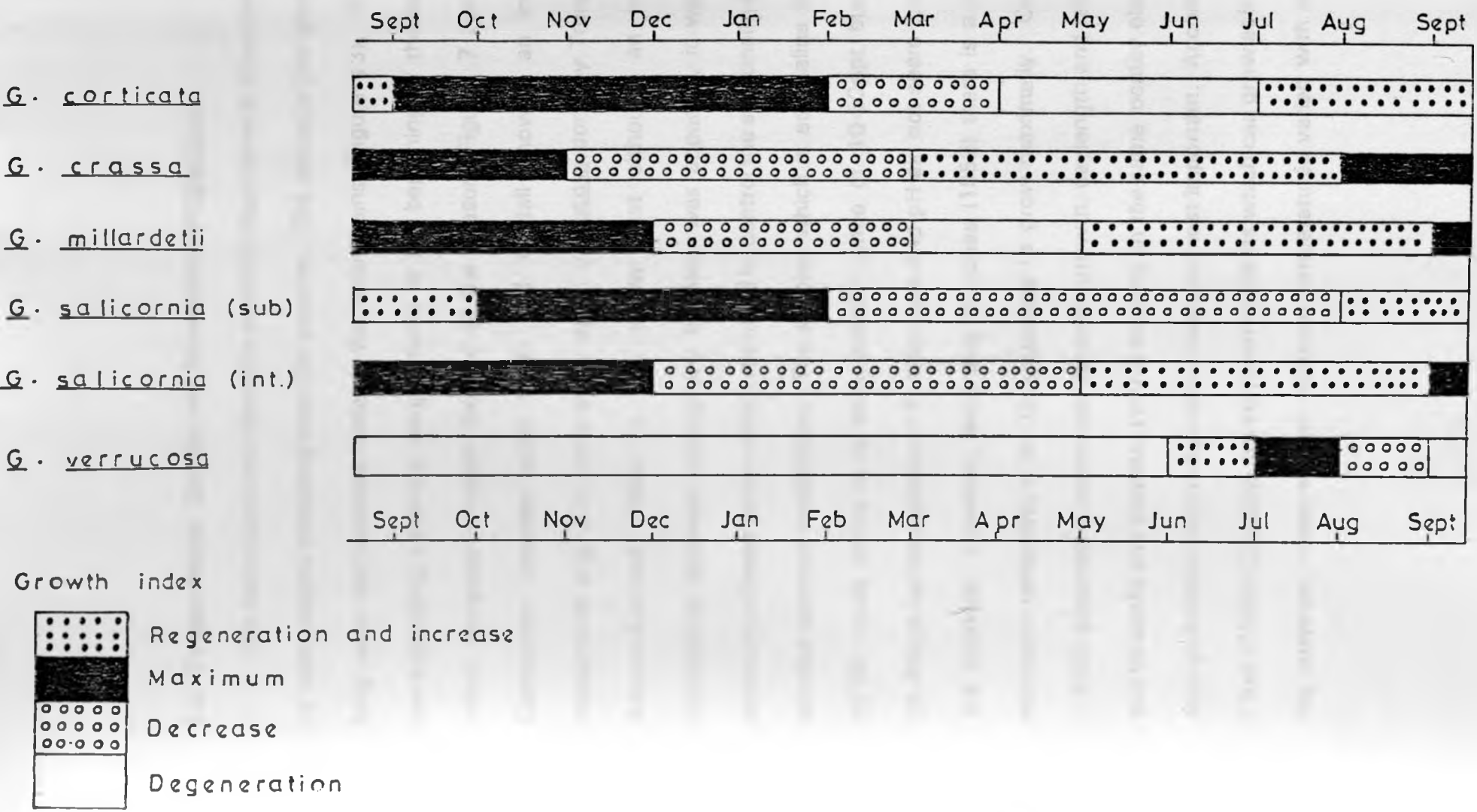



Fig 25 The pattern of seasonal standing crop of some Gracilaria species.

### 6.2. Temperatures, Salinity and Seasonality of Gracilaria

The period depicted above for maximal algal cover is characterised by calm waters prevailing over the country. The period is just after the long rains and extends through the short rains (Figure 13). It has corresponding moderate temperatures at the beginning of the season which increases towards the end of the season (Figures 7 and 26). Correlation between algal cover and rainfall showed an inverse relationship (Fig. 27). In a study on G, verrucosa done by Trono and Azanza-Corrales (1981) it was shown that although an inverse relationship between salinity and biomass was apparent, it was the pronounced wave action which appeared to control the seasonality of the species biomass production. The species which is euryhaline (Hoyle, 1975) could thrive in a wide salinity range of 10-50ppt but with 25-35ppt as the optimum. Temperature and pH did not seem to affect the biomass. However, according to Jones (1959) there is a critical minimum temperature for G. verrucosa to grow maximally. Conover (1964) observed a correlation between growth, daylength and radiation but Edwards and Kapraus (1973) working in the same locality observed that correlation with temperature was the most important. According to Chen (1989) Gracilaria are eurythermal plants which can grow at 5-30°C. He however, observed that optimum temperatures varied with species

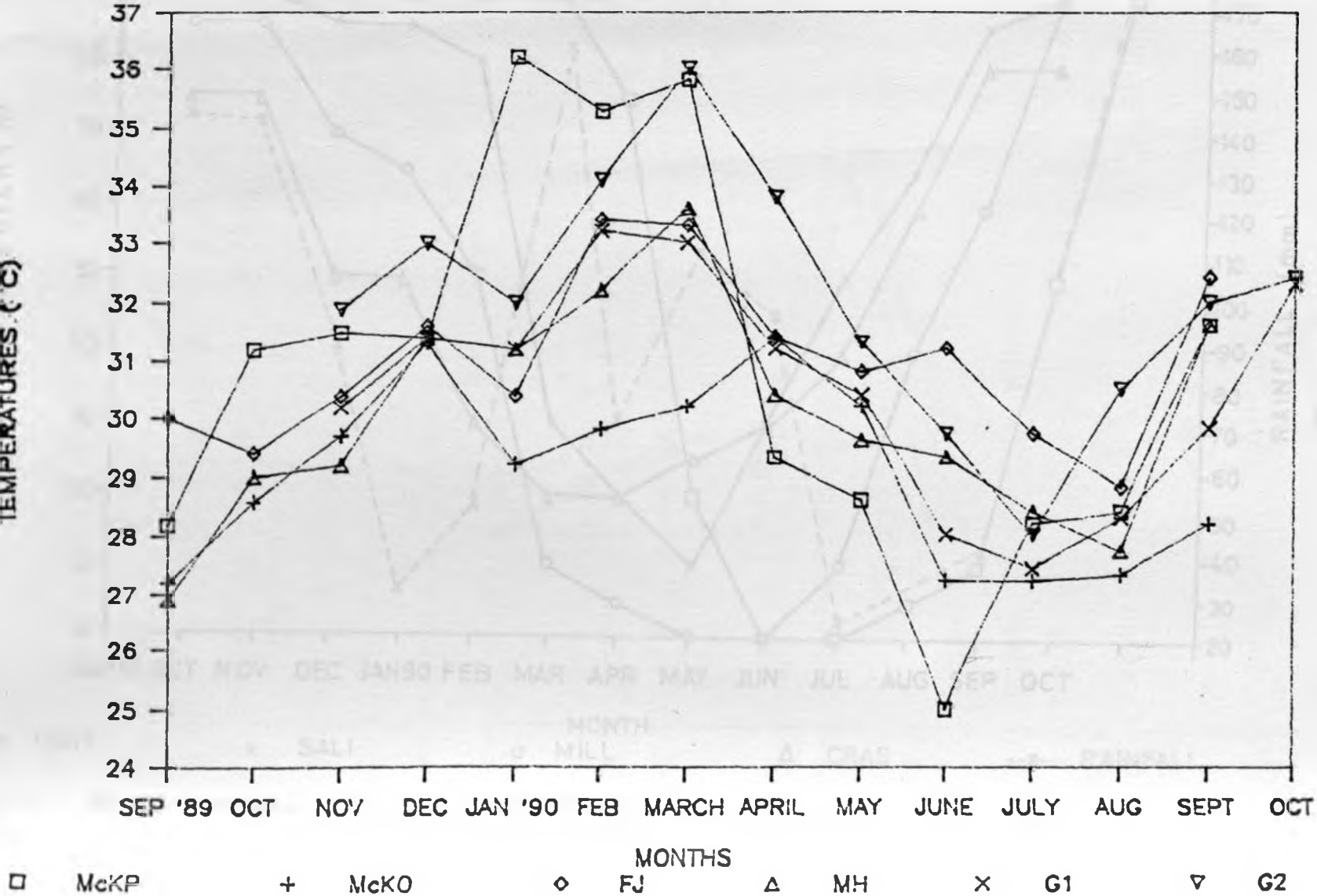
and in his study he reported that the optimum temperature for G. verrucosa is 15-25°C. This could offer an explanation as to the occurrence of this species only for a very short, cool part of the year when water temperatures as low as 23°C were recorded.

Gracilaria are euryhaline seaweeds. Under natural conditions they can grow within a range of 5.2-38.1ppt. Experiments and field surveys have shown that the optimum salinity is 11.3-30.1ppt where fresh water regularly flows in (Zheng et al., 1987). Bird and McLachlan (1986) also reported euryhalinity in the genus. The lowest salinity observed in this study was 24ppt while the highest was 38ppt. The abnormally high salinities observed during the month of April only and this is coupled by the effect of evaporation during low tide thus creating microclimates could have been due to evaporation during low tide thus creating a different microclimate within the intertidal zone. In this study seasonality of the seaweeds do not seem to have any special relationship with salinity and this may be explained by the fact that Gracilarias are euryhaline. However, observations made on G. millardetii showed that the plant tended to grow much better with a larger percentage cover in areas that had low salinities due to seepage from underground. Some of the pools had salinities as low as 22ppt.

Zheng et al., (1987) showed that the growth of seaweeds vary when they are planted at different depths of water. The study showed that Gracilarias can grow well in waters less than 1m deep where the sea water transparency is around 3m. In the present study the growth of Gracilaria, particularly G. corticata, was observed as deep as 5m at high tide. This could be attributed to the fact that transparency in this area is much higher (Figure 8).

# FIG.26: WATER TEMPERATURES OF STATIONS

DURING SAMPLING



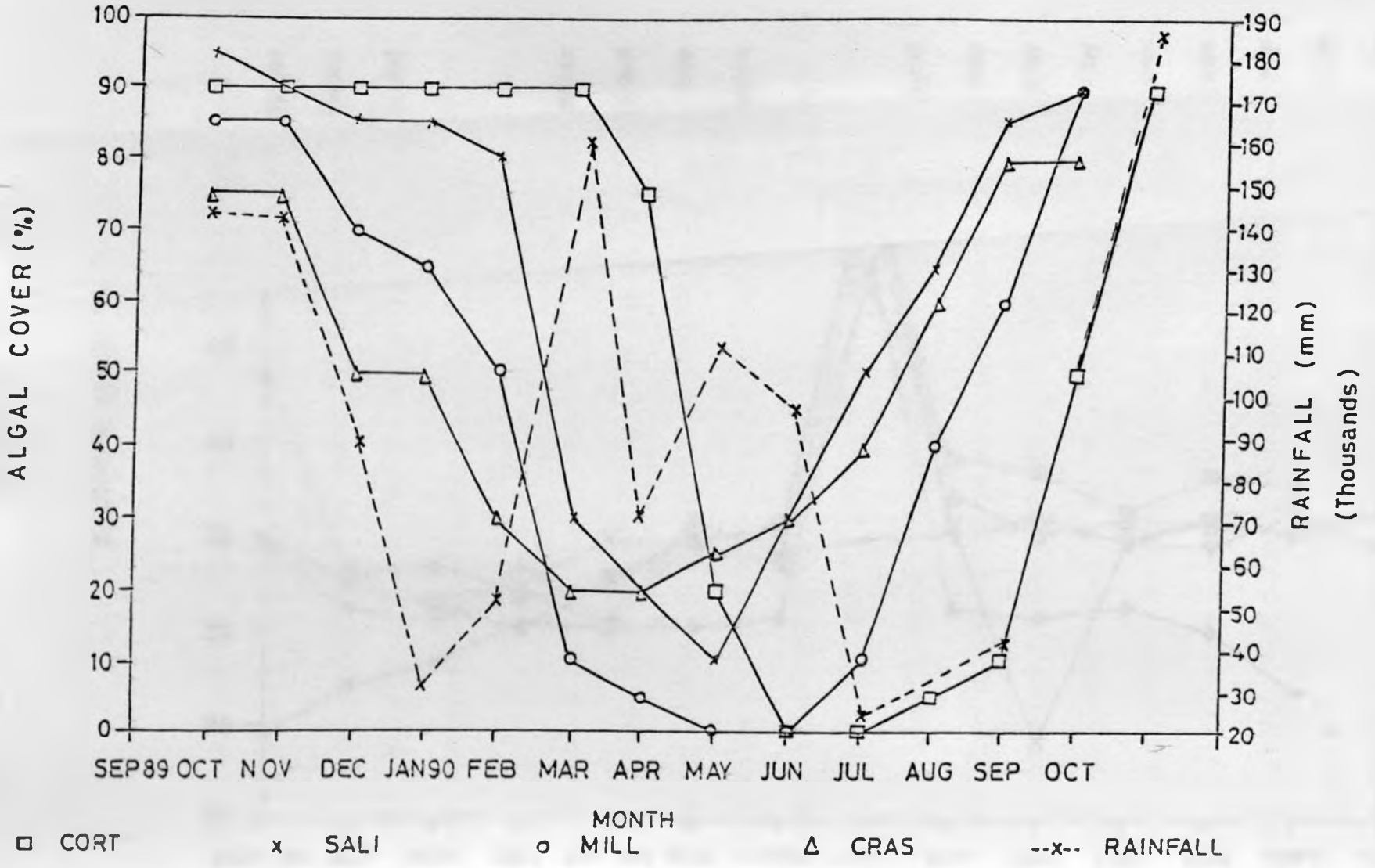
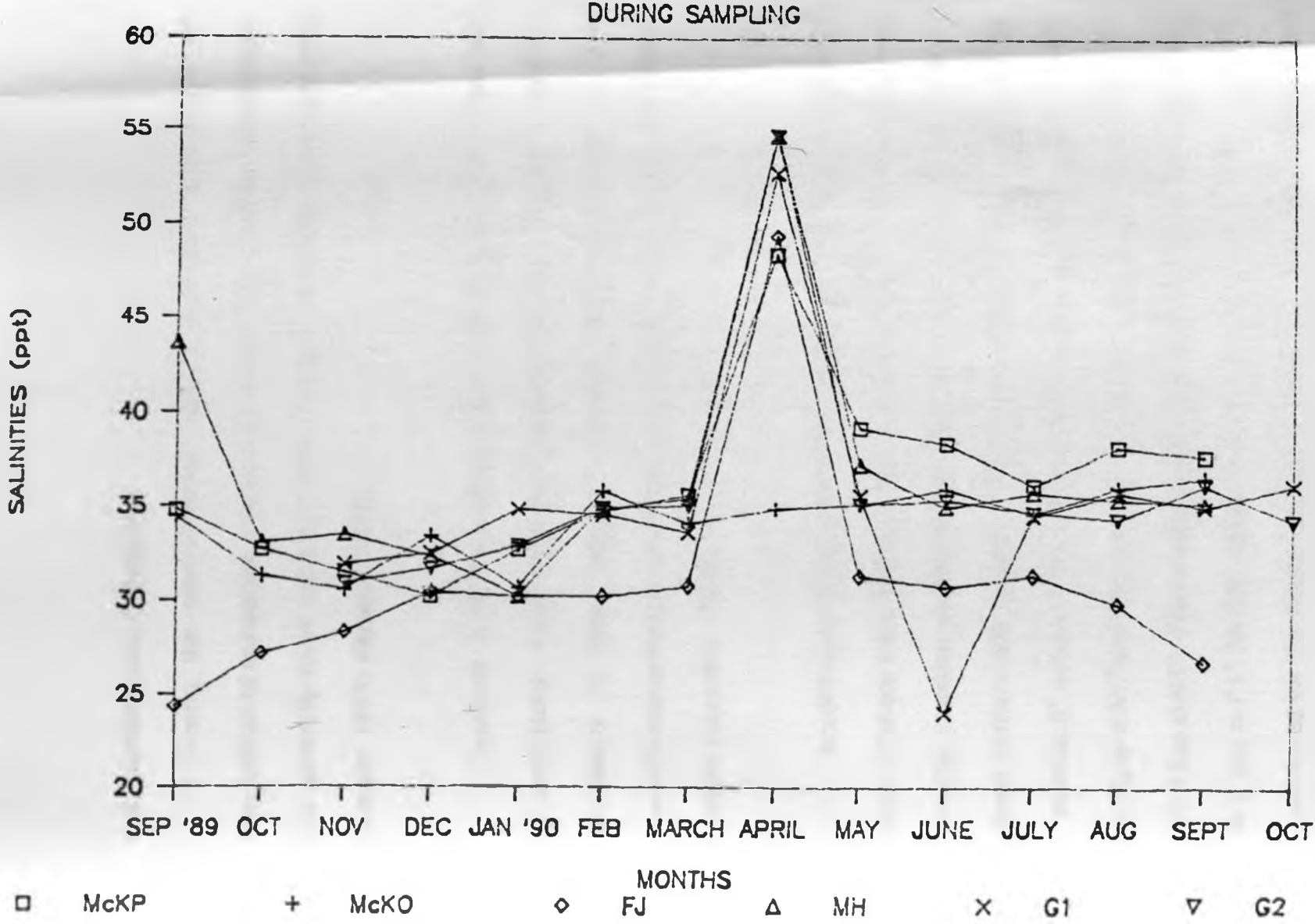



Fig. 27. Relationship between algal cover and rainfall.

# FIG. 28: SALINITY MEASUREMENTS



## 6.3. Nutrients and Seasonality

Among the environmental factors that limit productivity of macroalgae in temperate coastal waters, irradiance and/or temperature are generally more important than nutrient availability (Chapman and Craigie, 1977; Hanisak, 1979).

However, in the tropical and subtropical waters nutrient limitation is considered more important because of generally lower nutrient availability in these waters. Nitrogen and phosphorus are two macronutrients that generally limit macroalgal growth in nearshore marine waters (Lapointe, 1989).

According to Düing (1970) strong winds (South-East monsoons) blow offshore from Africa during June/July/August. These winds may directly transport surface waters away with a resulting replacement by deep nutrient-rich waters. The nitrate distribution as reported by "Atlantis II" survey in the Western Indian ocean during the SE monsoon is 25$\mu$g at N $l^{-1}$ and the equatorial area is distinguished by values between 10-15$\mu$g at N $l^{-1}$. The corresponding phosphate phosphorus for the region is 0.2$\mu$g at P $l^{-1}$ (McGill, 1973). Values of nutrients higher than the above mentioned for the region are due to coastal conditions as evidenced by

observations made during this study (Figures 29 - 36). Apart from upwelling, other sources of nutrients in the sea which is also important in this study is rainwater discharges which bring nutrients washed off from land. Measurements for this study were taken during low tides. Some of the sampling stations like Fort Jesus and Mombasa Hospital are heavily influenced by human activities in the form of hospital and domestic discharges onto the shores, and therefore resulted in very high levels of nutrients in the intertidal pools.

Relating the above nutrient availability information to seasonality of the plants under study it is logical that the seaweeds do make use of the nutrients made available during the SW monsoons for maximum growth in the subsequent months as depicted in the study. Furthermore, Thomas et al. (1987) carried out a study in which they showed that macrophytes growing higher up in the intertidal zone are adapted to higher levels of desiccation and shorter periods of immersion in seawater containing nutrients by increasing their nitrate and ammonium uptake upon submersion after a period of desiccation. They reported that their uptake rates were an estimate of short-term uptake and not steady-state nutrient procurement since in their study they had short duration of uptake experiments using higher concentrations than the normal ambient

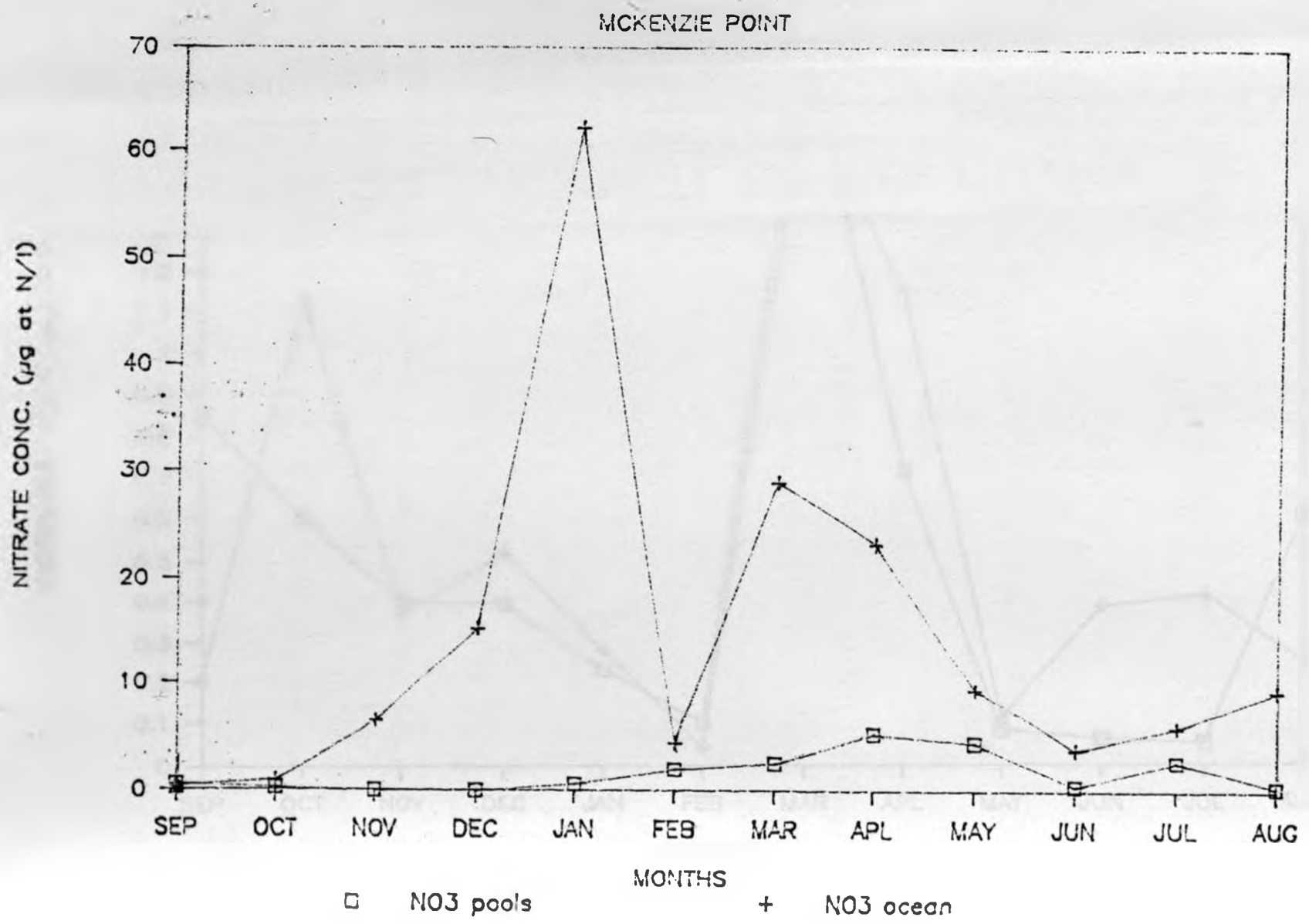
concentrations. Short-term uptake can be important if brief 'patches' of higher concentrations occur, and if the amount of nutrient taken up during these 'patches' is significant to long-term nutrient procurement for growth. As established in this study most of the Gracilaria species grow in the eulittoral zone and since members of the genus require high levels of nutrients (Santelices and Doty, 1989), the phenomenon described by Thomas et al. (1987) could be enhancing their nutrient uptake from the nutrient enrichments brought about by coastal effects as seen in some of the stations where nutrient measurements were taken.

Furthermore, Gracilaria, like most seaweeds, can accumulate nutrient reserves within the tissues of the thallus (McLachlan and Bird, 1986) thus enhancing nutrient uptake from the enriched waters. Internal reserves of nitrogen accumulate in several pools, both as inorganic salts and organically bound compounds. These are reported to be available for growth when ambient levels of nitrogen in surrounding waters become reduced or depleted.

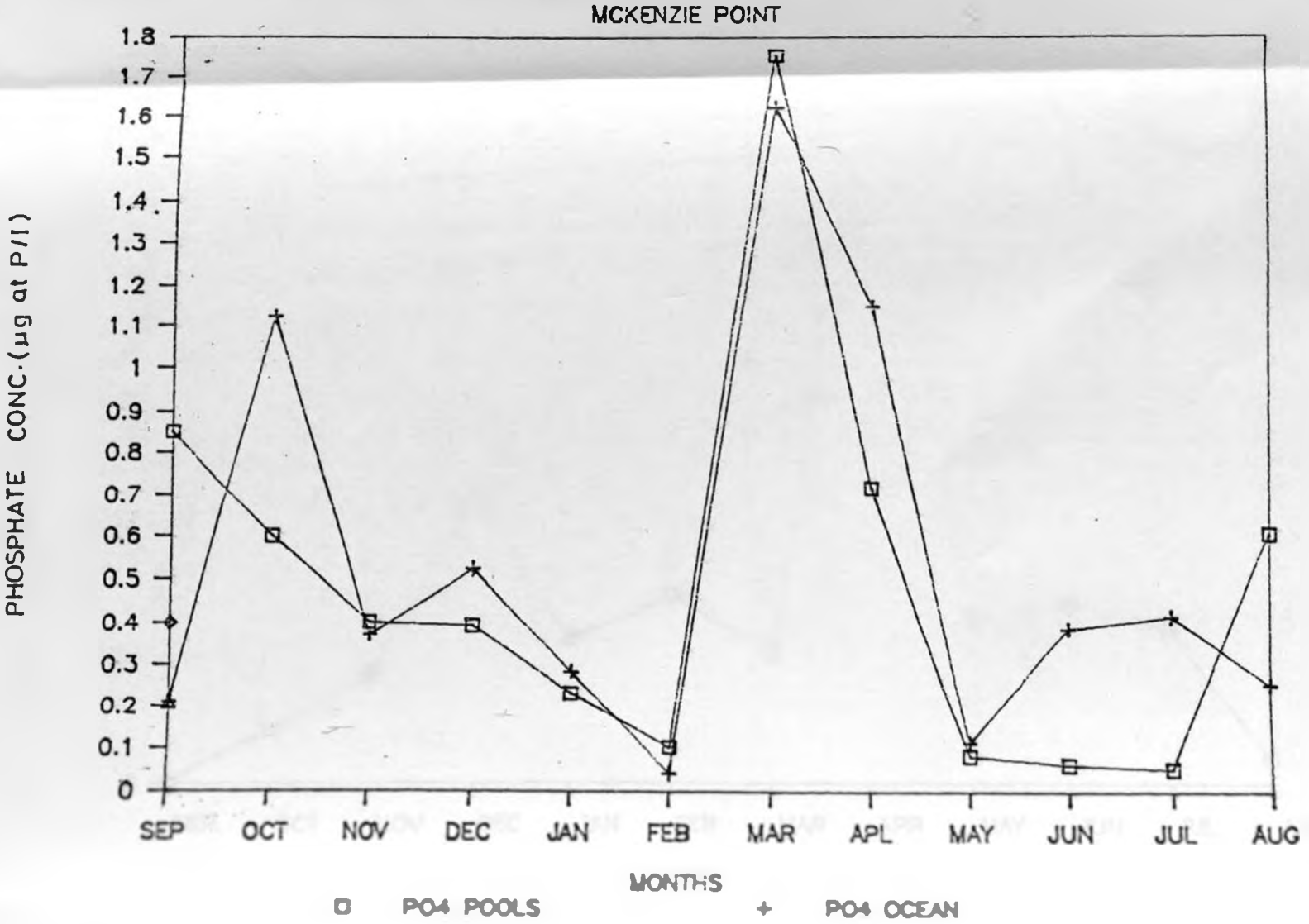
Primary productivity studies at Gazi station recorded the highest average net productivity in the period covering June to October 1990 with the highest in June (97.46mg $C/m^2/hr$) while on average the period

covering March-April had a lower value (lowest in April 7.46mgC/$m^2$/hr) (Wawiye: personal communication). This goes further to support the theory of nutrient increase during the SE monsoons. In conclusion it may be noted that seasonal abundance of the different Gracilaria species vary with time and station but in general one main peak season can be recognised. These observations indicate the existence of a regular seasonal succession in the regeneration , maximum growth and decline of the different species along the Kenya coast.

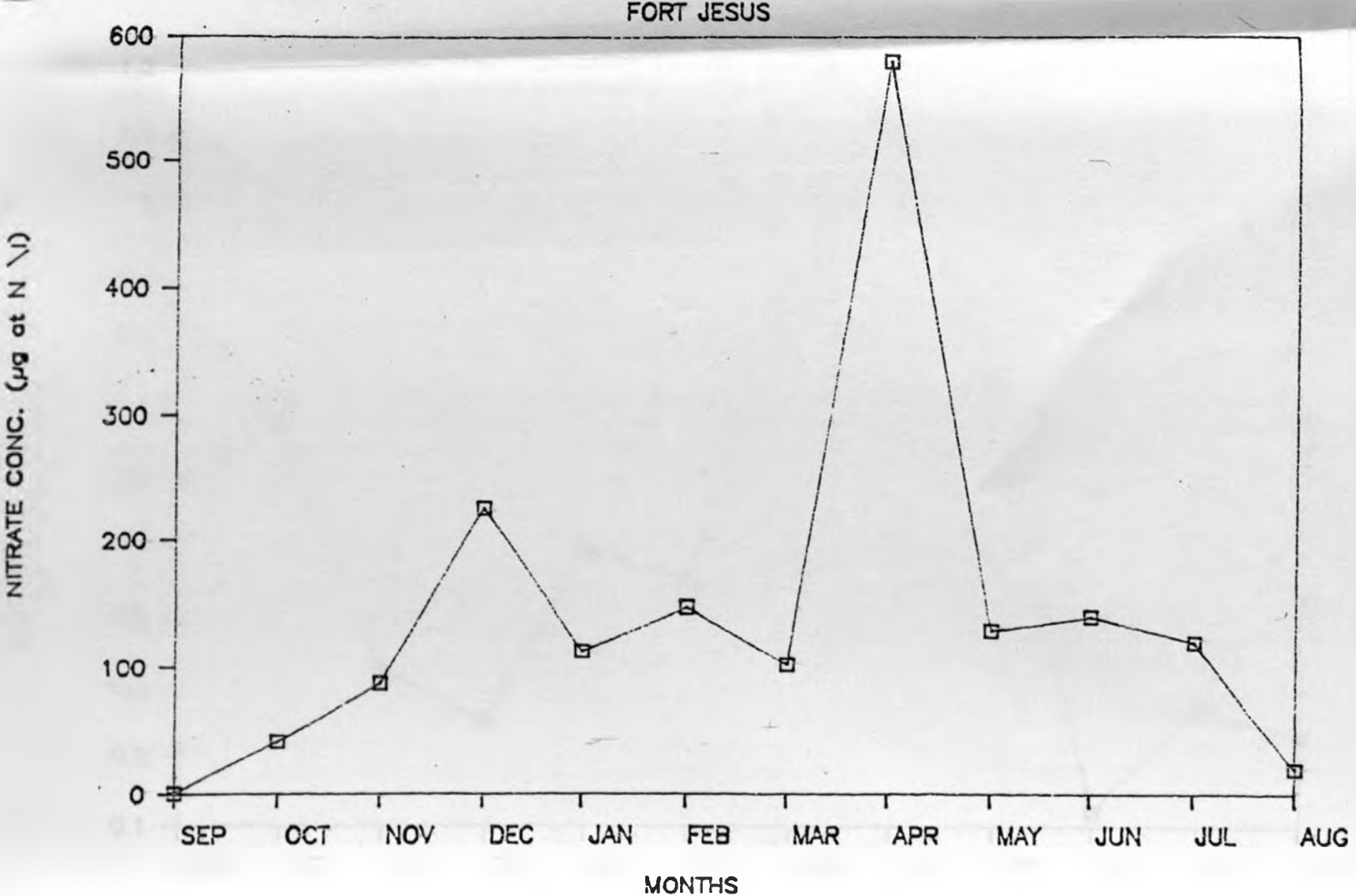
# FIG. 29: NITRATE CONCENTRATIONS



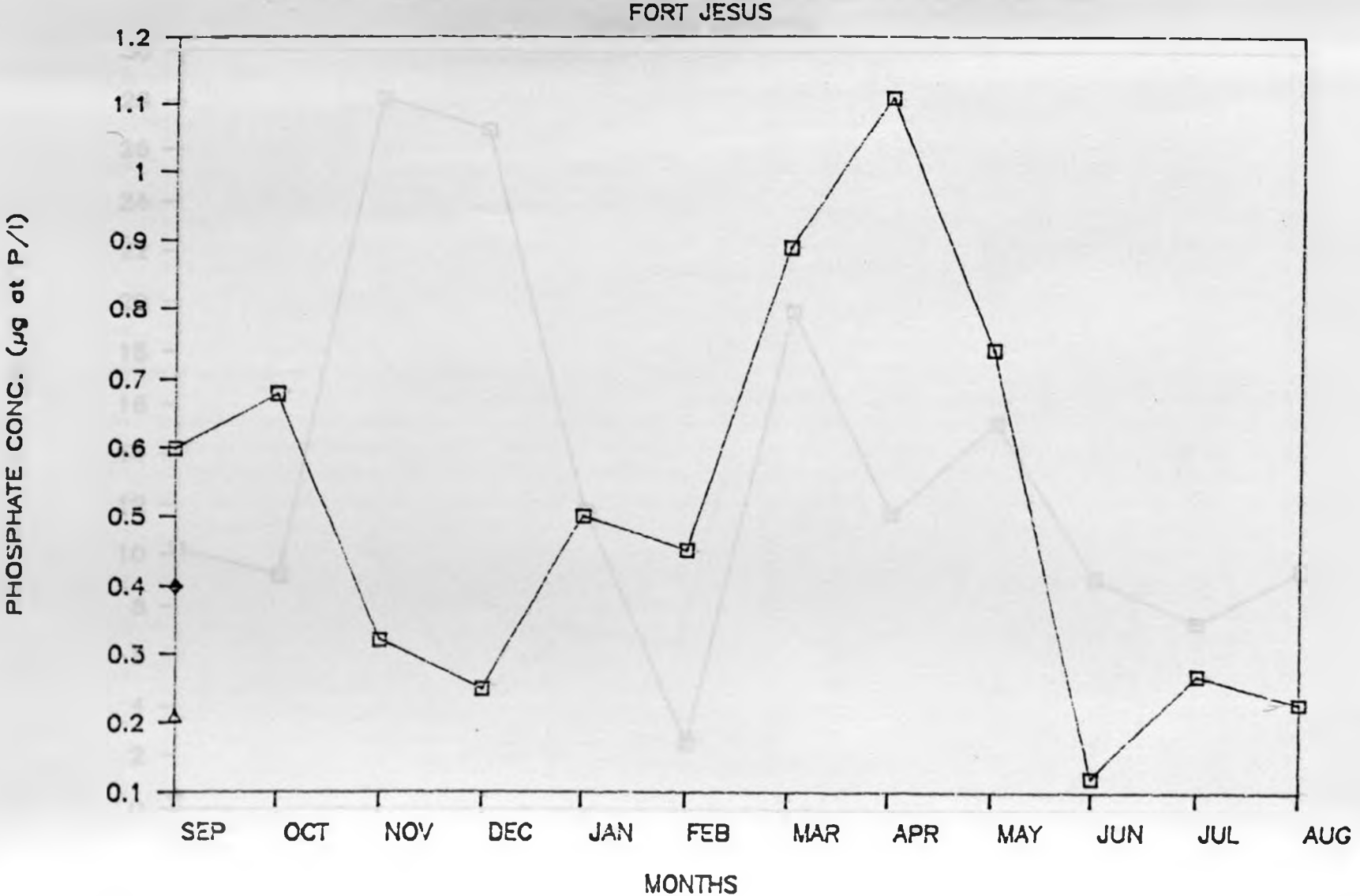
# FIG. 30: PHOSPHATE CONCENTRATIONS



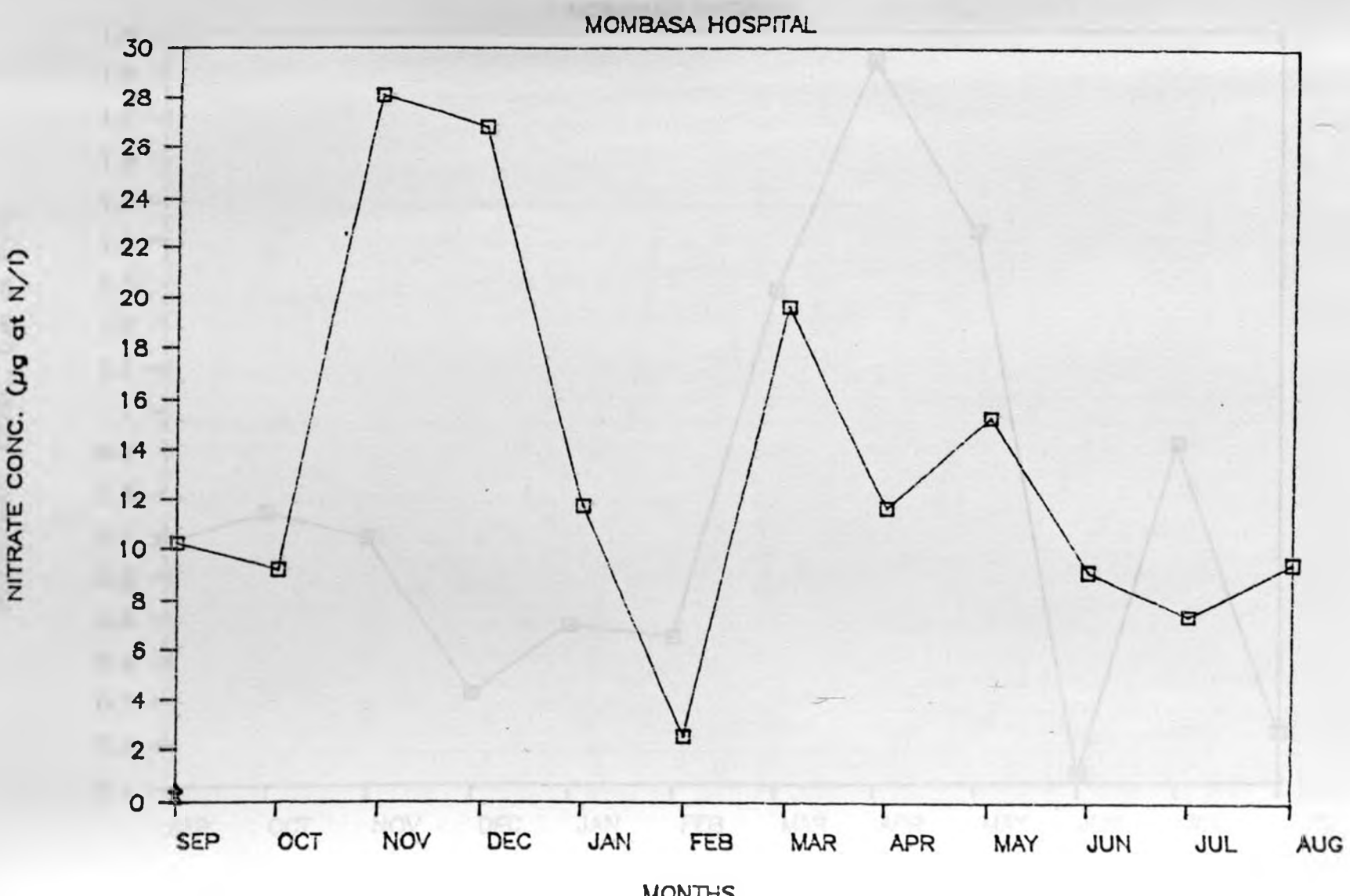
# FIG. 31: NITRATE CONCENTRATIONS



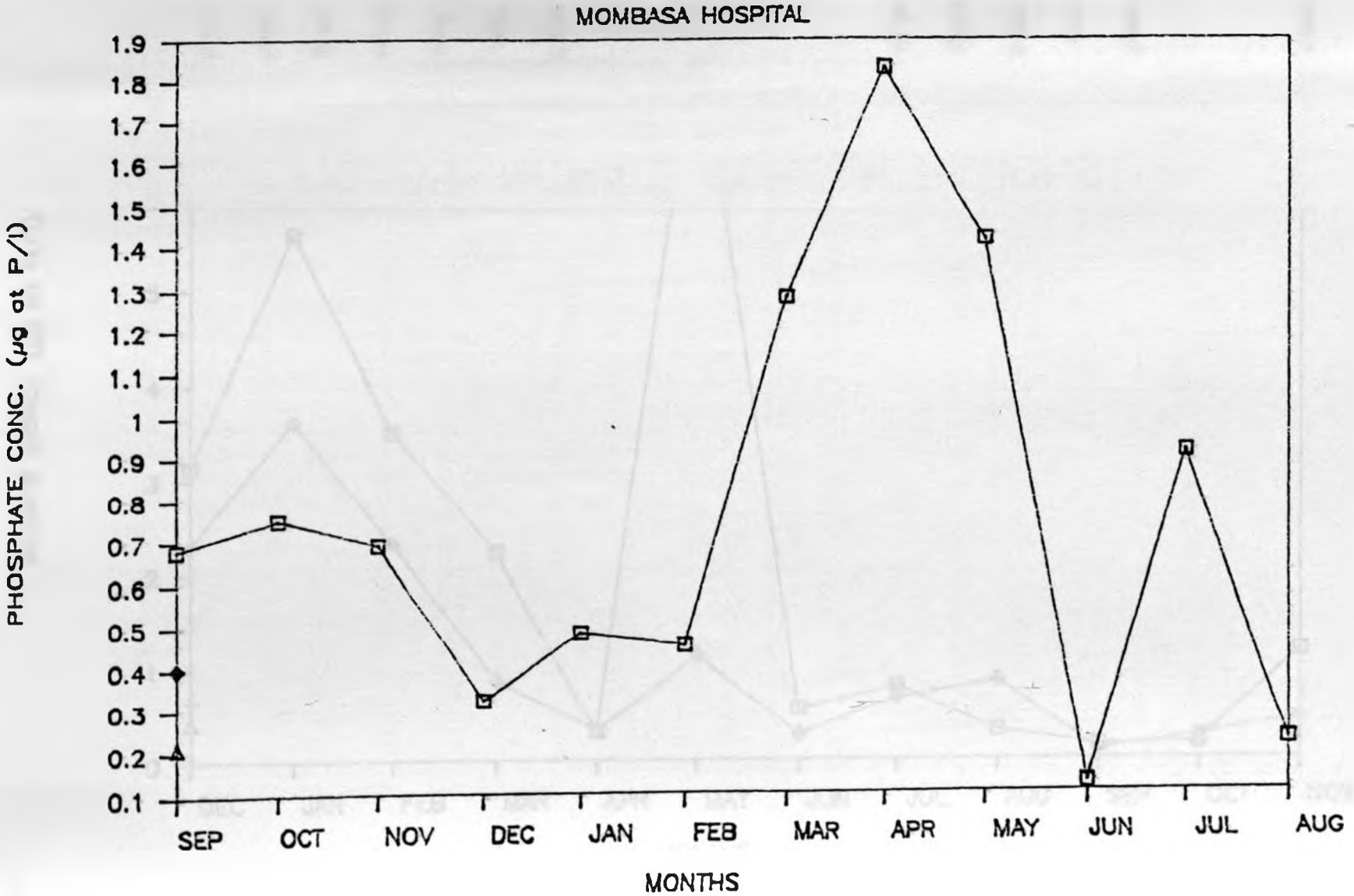
# FIG. 32: PHOSPHATE CONCENTRATIONS



# FIG. 33: NITRATE CONCENTRATIONS



# FIG. 34: PHOSPHATE CONCENTRATIONS



# FIG. 35: NITRATE CONCENTRATIONS

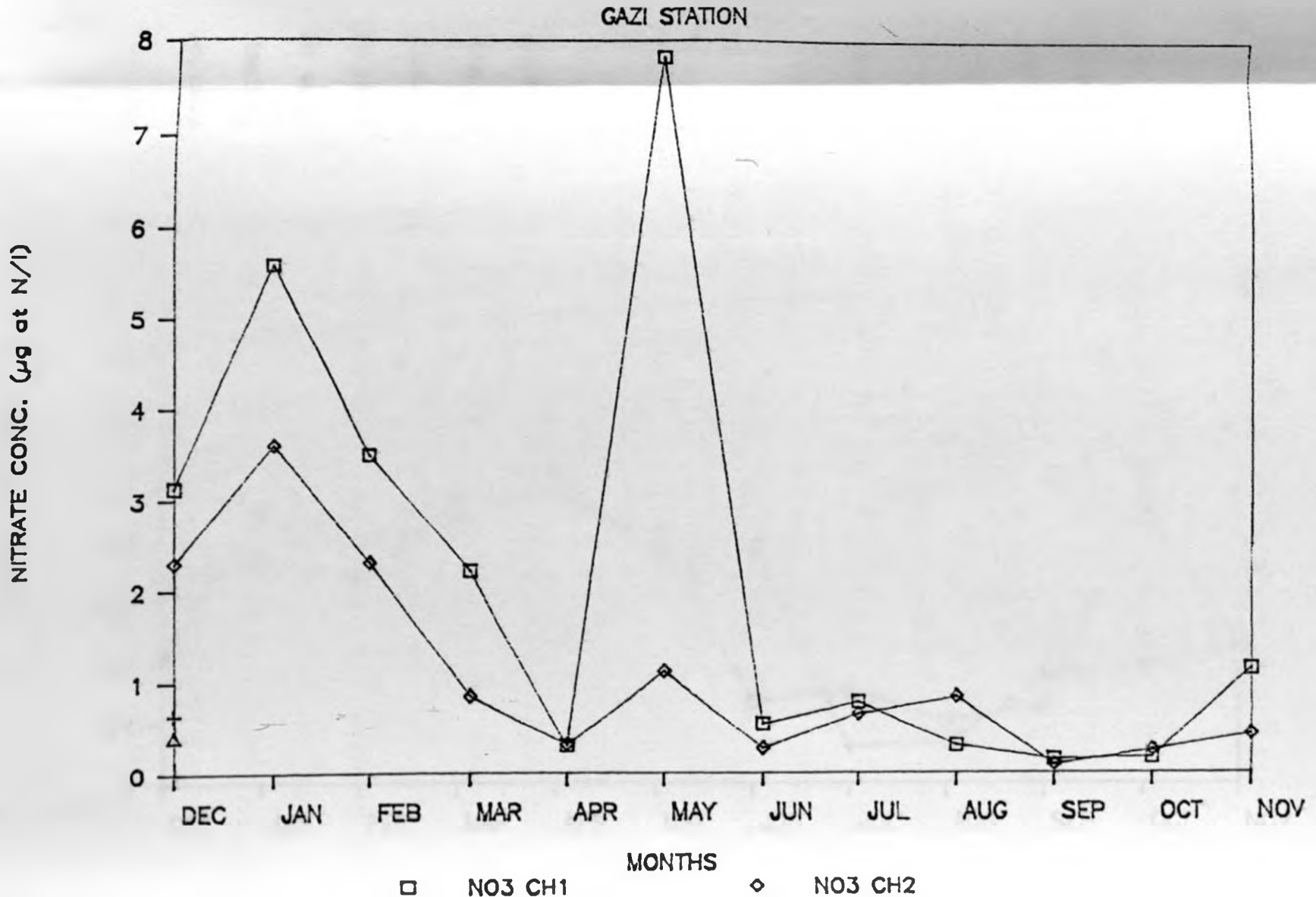
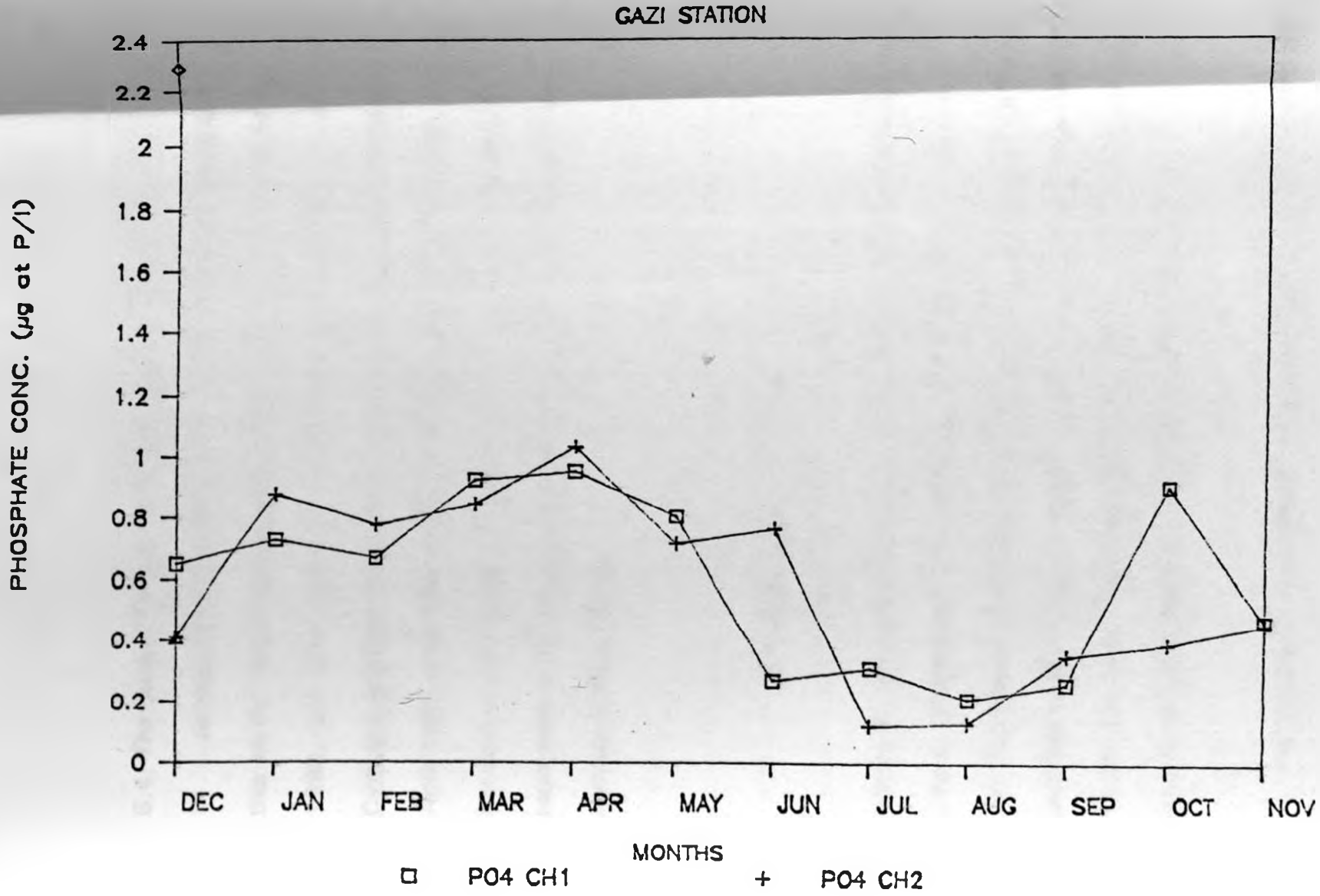


FIG. 36: PHOSPHATE CONCENTRATIONS



## 6.4. The Growth Pattern of G. corticata

An experimental population was identified at McKenzie station for this study. Observations were made from the first week of August, 1990, just when growth of new thalli were observed on stems of Cymodocea ciliata. The growth was studied up to the second week of April 1991, when the whole population degenerated and was no more. For each 4-week growth period, the growth rate of harvested plants was expressed as the mean relative growth rate (R) in % per week using the formula in Hunt (1978).

$$R = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1} \cdot 100$$

Where $W_2$ is the fresh weight at time $t_2$ and $W_1$ is the fresh weight at time $t_1$. The growth curve obtained for this study is as shown in figure 37. The fastest growth rate for G. corticata was recorded during the 8th and 9th week of growth where the relative growth rate recorded was 53.2% per week. The slowest growth rate of less than 5% per week was recorded during the last 12 weeks of growth.

The population investigated had a growth period of 8-9 months and

carpospores were observed during the 3rd and 4th months of growth. By the 5th month all the spores had been shed. The period taken from the time spores were formed to the time they sprouted out into new growth was 8-9 months. Studies done by Bird et al., (1977) on the life history of Gracilaria in vitro revealed that detached plants showed the greatest reproductive potential and that their reproductive maturation occurred in 8-10 weeks after spore germination. In the present study reproductive maturation occurred in 11-12 weeks after spore germination. It is important to note here that this pattern of growth may not be true for all populations of G. corticata along the Kenyan coast. During the study it was noted that small populations of the same species were growing at other stations during periods when the McKenzie population had completely degenerated. Hence, the pattern of life cycle may vary from one population to another depending on geographic location. This pattern may also vary depending on ecotypes. This is supported by the fact that differences in periods of fertility was observed between the McKenzie and Gazi populations. Whereas the former had its population fertile from November through December the latter had its population fertile during the months of November through to April. The Gazi population therefore had a longer period of fertility. These findings are in accord with those of Anguilar-Rosas and Galindo (1989), who

reported different periods of fertility for different populations of Sargassum muticum.

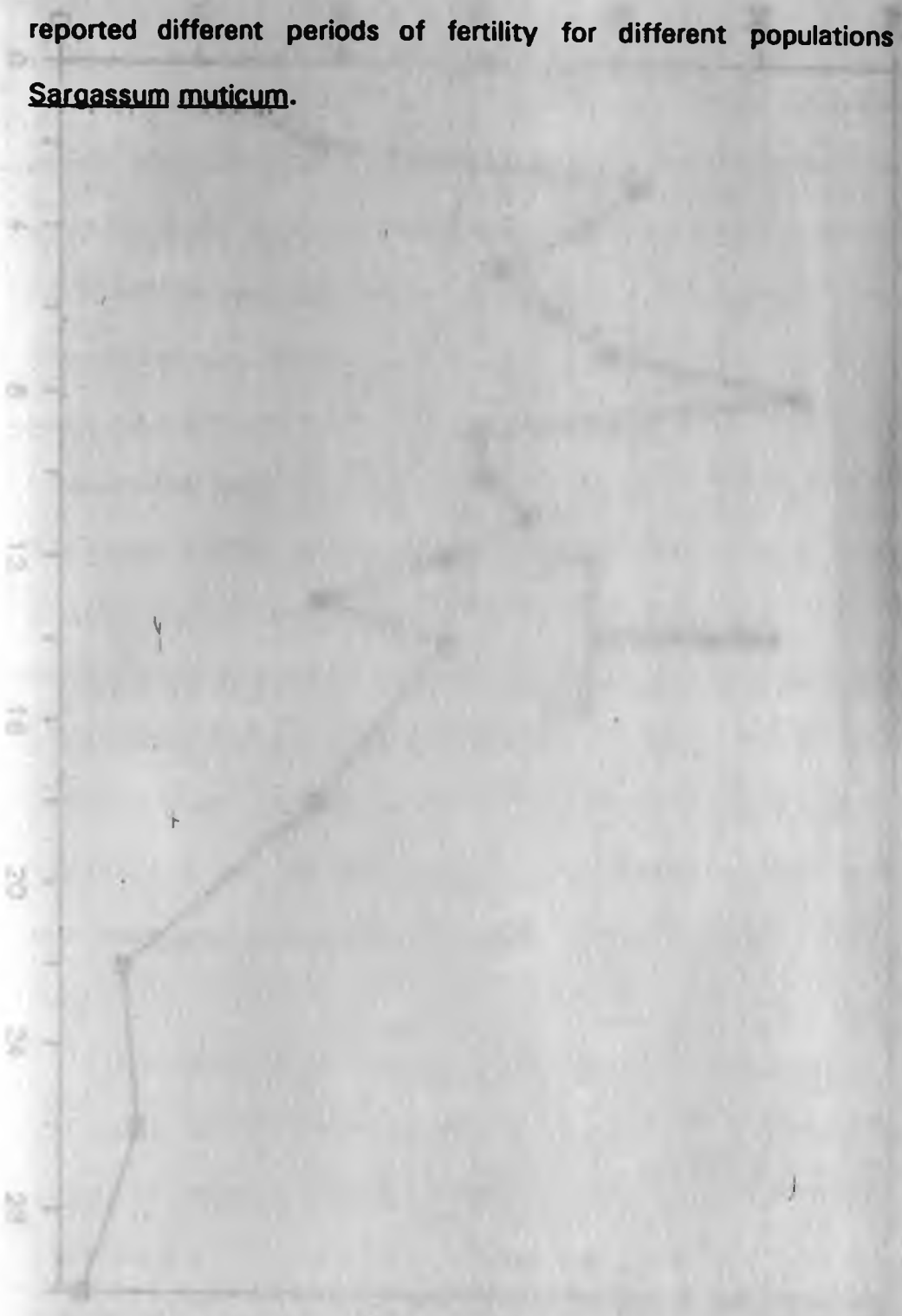
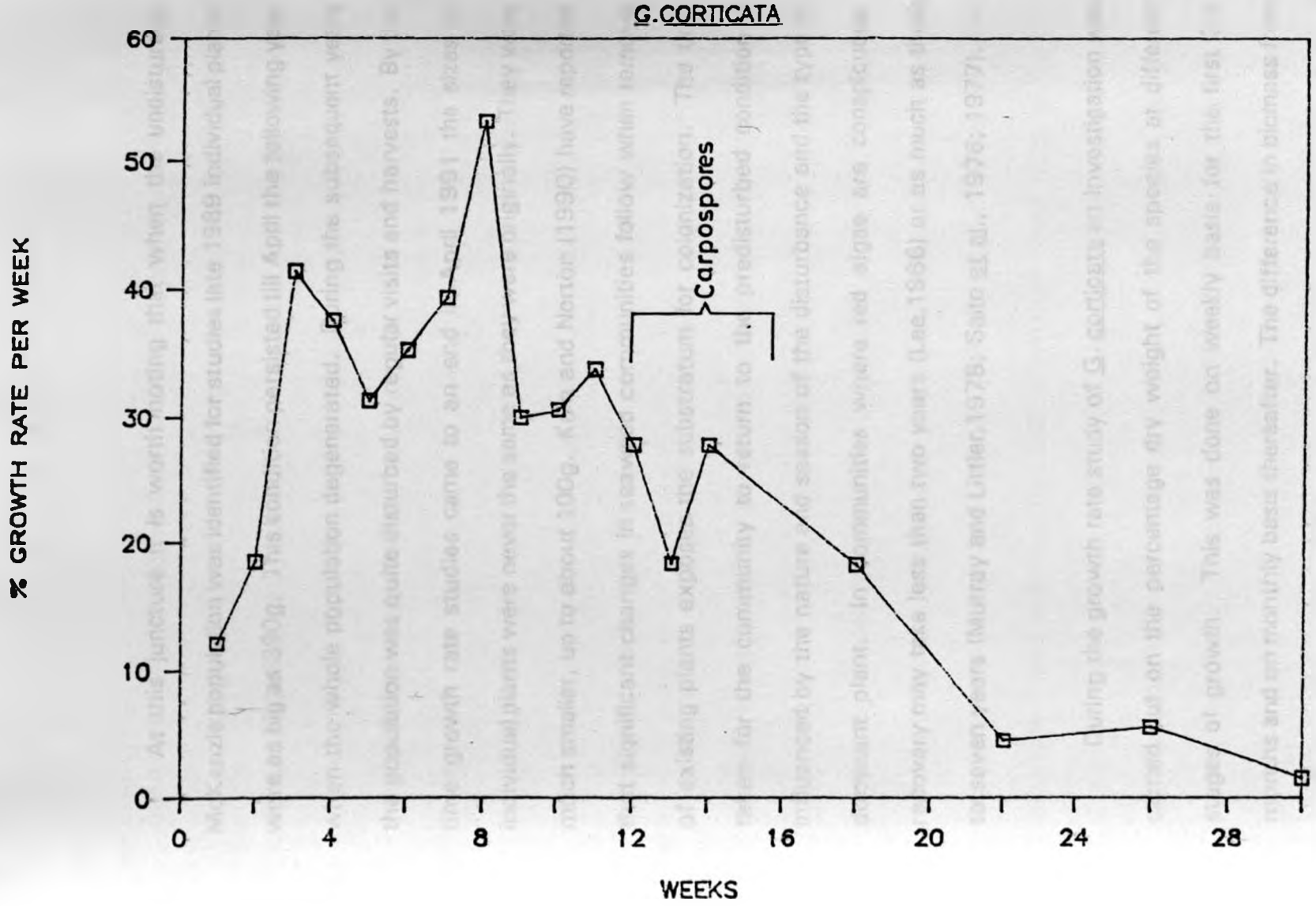


FIG. 37: WEEKLY GROWTH RATE

# FIG. 37: WEEKLY GROWTH RATE



At this juncture it is worth noting that when the undisturbed McKenzie population was identified for studies late 1989 individual plants were as big as 390g. This condition persisted till April the following year when the whole population degenerated. During the subsequent years the population was quite disturbed by regular visits and harvests. By the time growth rate studies came to an end in April 1991 the sizes of individual plants were never the same as they were originally. They were much smaller, up to about 100g. Kain and Norton (1990) have reported that significant changes in seaweed communities follow when removal of existing plants exposes the substratum for colonization. The time taken for the community to return to the predisturbed condition is influenced by the nature and season of the disturbance and the type of dominant plant. In communities where red algae are conspicuous, recovery may take less than two years (Lee,1966) or as much as three to seven years (Murray and Littler,1978; Saito et al., 1976; 1977).

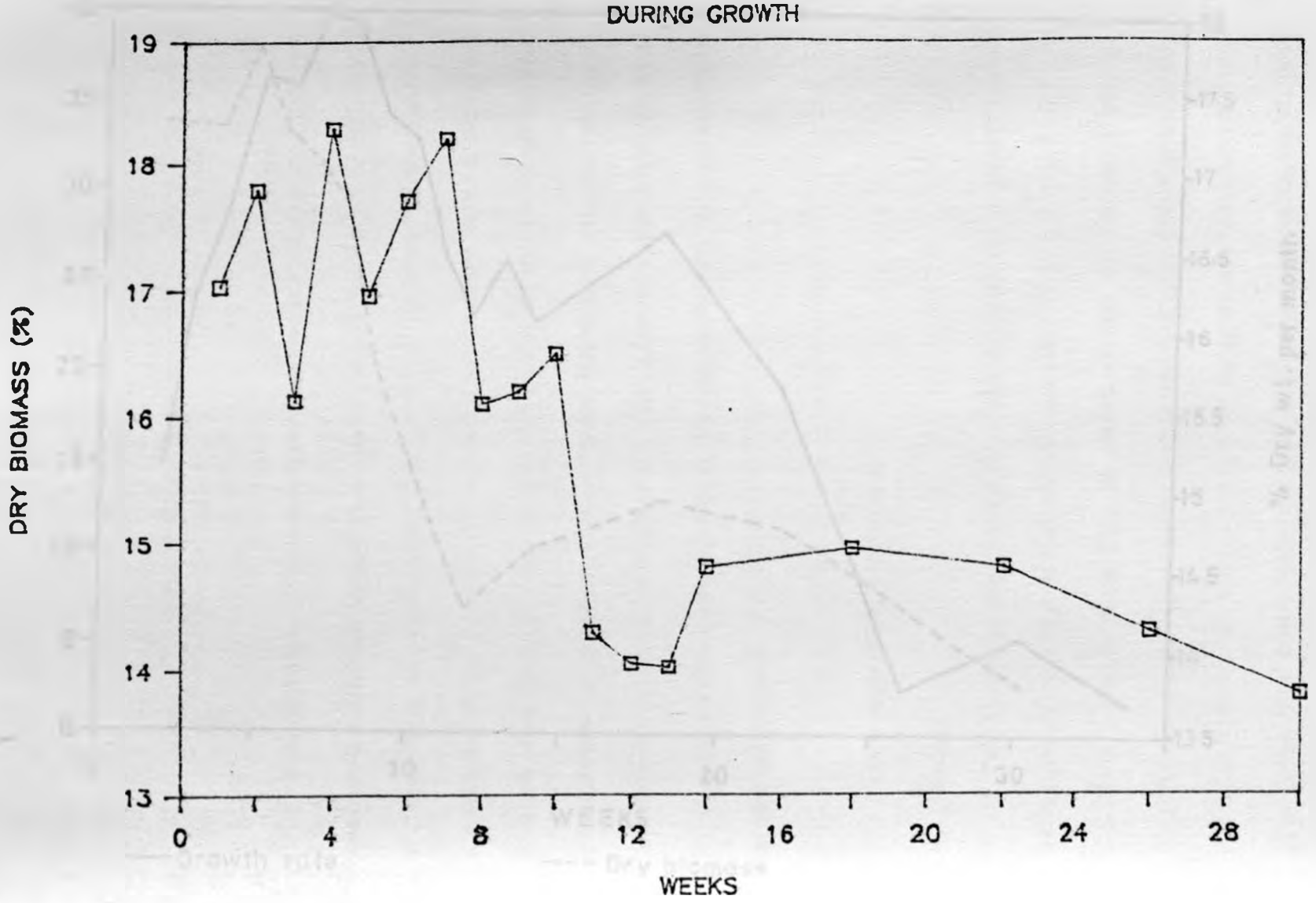
During the growth rate study of G. corticata an investigation was carried out on the percentage dry weight of the species at different stages of growth. This was done on weekly basis for the first 3½ months and on monthly basis thereafter. The difference in biomass from one week to the other did not change significantly. However, a trend

was shown (Figure 38), whereby the early stages of growth i.e. period before maturity, had a significantly higher biomass than the subsequent period ($p=0.01$). For the first 11 weeks the dry weight fluctuated between 16 and 18.2% of the fresh weight while during the latter period it fluctuated between 14 and 15%.

Correlating dry weight and growth rate directly may not give the correct relationship in this study since the ash content was not determined. However, McLachlan and Bird (1986) reported the dry weight of species of Gracilaria in their study as ranging from 10-12% of the fresh weight and they also indicated that figures between 8% and 20% had been reported in other investigations. These variations, they suggested, indicate variations in environmental conditions. They further reported an inverse relationship between percentage ash and dry weight, thus reflecting various levels of accumulated organic matter. In some instances it was observed that ash constituted about 50% of the dry matter whereas in others it fell to 25%. A negative correlation between ash and growth, and a direct relationship between organic matter and growth has been reported by Lapointe (1981). By infering from the two reports, it would therefore appear that the higher values of dry weight during the first phase of growth (Fig. 39) are due to organic matter

synthesis. This is contrary to Friedlander et al. (1985) who suggested that during good growth seaweeds absorb more water than they synthesize organic compounds or absorb minerals. The differences in the current results and that of Friedlander et al. (1985) could be attributed to the fact the present study was done in natural habitat where a lot of factors come into play while they did their study under controlled experimental conditions.

FIG.38: % DRY WEIGHT OF G. CORTICATA



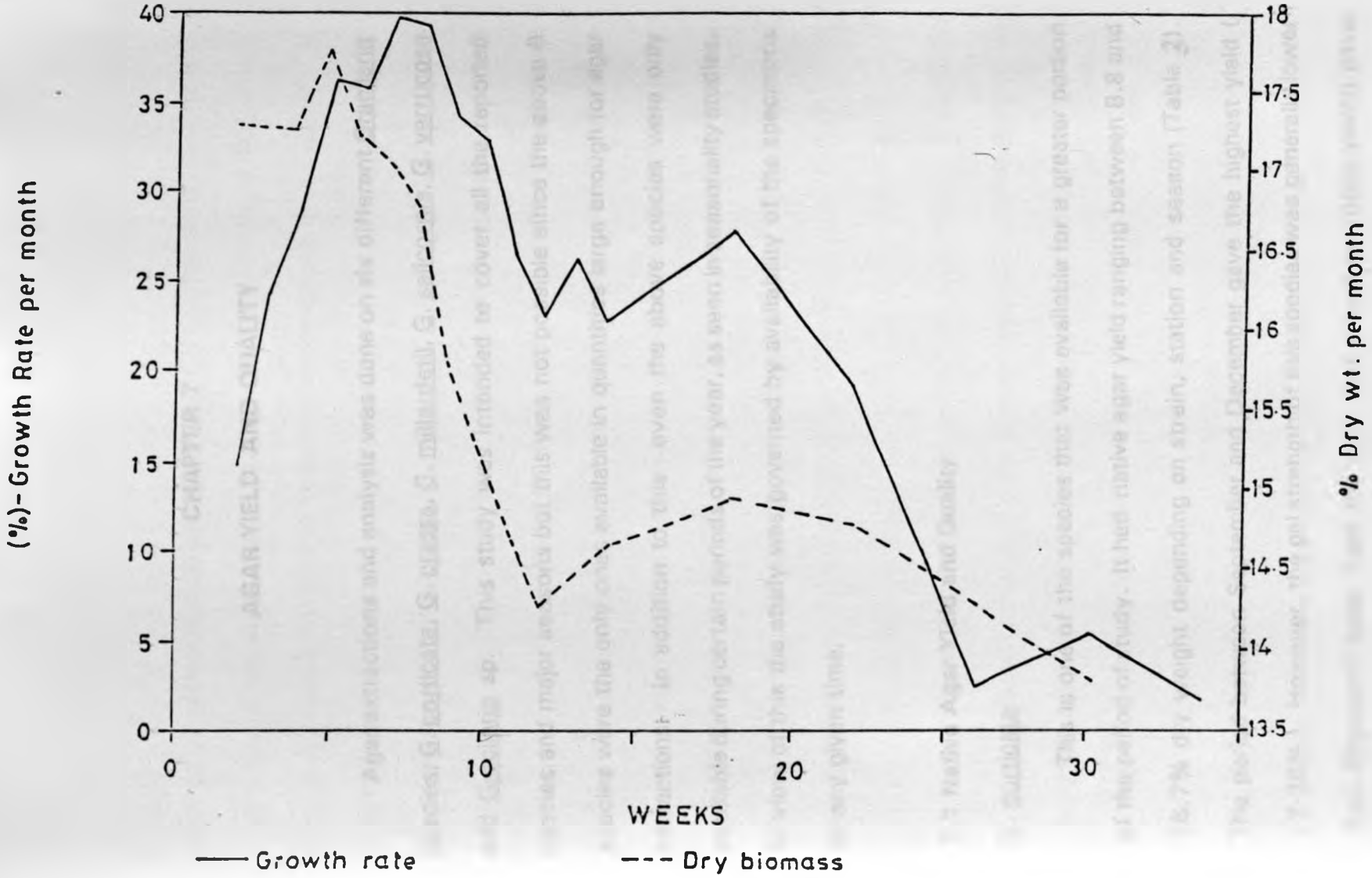



Fig.39. Relationship between growth rate and dry biomass of G. corticata

# CHAPTER 7

# AGAR YIELD AND QUALITY

Agar extractions and analysis was done on six different Gracilaria species; G. corticata, G. crassa, G. millardetii, G. salicornia, G. verrucosa and Gracilaria sp. This study was intended to cover all the reported species and major seasons but this was not possible since the above 6 species were the only ones available in quantities large enough for agar extractions. In addition to this even the above species were only available during certain periods of the year, as seen in seasonality studies. In view of this the study was governed by availability of the specimens at any given time.

## 7.1 Native Agar Yield and Quality

G. corticata

This is one of the species that was available for a greater portion of the period of study. It had native agar yield ranging between 8.8 and 26.7% dry weight depending on strain, station and season (Table 3). The period between September and December gave the highest yield ( 17-26% ). However, the gel strength of this species was generally lower than 60gm/cm$^2$ apart from the type that grows on rocks which gave

readings of between <60 and 80gm/cm$^2$. The apparatus used for this measurement could only take readings that were above 60gm/cm$^2$. Though it was not possible to measure gel strengths below 60gm/cm$^2$ it was observed that gels from plants harvested when the majority of the plants were cystocarpic were so weak that their gels just formed thick mass at their gelling temperatures. The gelling temperatures of this species had a range of 29-35°C. Agar from older plants generally had lower gelling temperatures than from younger plants. Of the McKenzie and Gazi plants the ones that were harvested when the majority were cystocarpic exhibited significantly lower gelling temperatures than the rest. Melting temperatures ranged between 79 and 90°C showing no specific pattern.

Agar from the alkali-treated plants had significantly lower yield than the native one though the gel strength and gelling temperatures did not change significantly ( Table 10). The gel strength did not improve even in the alkali-treated agar.

G. crassa

This species had native agar yield ranging from 12-25% dry weight depending on season. The period between September and October gave

the highest yield, ranging between 23 and 25%. Analysis of native agar gave gel strengths varying between 125 and 205gm/cm$^2$ and the best gel strength came from August-September-October harvests. It is to be noted here that plants that were harvested from an area that was exposed only at extreme low tides had one of the highest yields but its gel strength was quite low compared to the harvests of the same season from plants that were exposed at low tides everyday. This population was only about 20m away from the other population higher up the shore. The gelling temperatures of this species was one of the highest, ranging between 36 and 37°C and these showed no significant variation from one season to the other. The melting temperatures were quite high, ranging from 91-94°C with a tendency of higher temperatures for gels that had higher gel strengths. Table 4.

Agar from alkali-treated plants yielded significantly stronger gel, 198gm/cm$^2$ as compared to the native agar, 125gm/cm$^2$. However, the gelling temperature did not change significantly and the yield was much lower than the native yield. The gel strength of the alkali-treated agar, on the other hand, did not improve significantly.

G. millardetii.

This species was available in quantities large enough for extractions only for a very short period of the year. However, agar extractions from the periods when it was available showed a relatively higher yield during September-October. Its agar yield ranged from 8.7-17.2% dry weight. Analysis of its gelling temperatures showed a variation between 30 and 34°C, its highest temperature corresponding with the period of the lowest agar yield. Its melting temperatures ranged between 86 and 92°C with the highest temperature corresponding to the highest gel strength. The species had one of the lowest gel strengths ranging from $<60 gm/cm^2$ to $99 gm/cm^2$. The highest gel strength was also recorded during the month of lowest yield.

Table 5.

Agar from alkali-treated plants did not show any significant difference from native agar in terms of gel strength and gelling temperature but the yield was lower. Even the alkali-treated agar showed no improvement in the gel strength.

G. salicornia

The species was one of the most commonly available for agar

extraction over a relatively larger portion of the year. In terms of agar yield it is one of the poorest of the species studied with yields ranging from 8-16% dry weight. There is, however, a marked distinction in terms of yield versus ecotypes. The four groups shown in Table 6 mark the different types. On the whole, the sublittoral type (Gazi) gave higher yields than the intertidal types. Of the intertidals the type that grow as clumps on the rocky-muddy reef flats (Shelly beach) gave higher yields than the ones that grow in shallow pools or directly on exposed rocky surfaces as cushions (McKenzie). No clear pattern of yield versus seasonality has emerged out of this study. Of all the species studied G. salicornia has shown the biggest variation in terms of gelling temperature, a range of 33-38°C. Its melting temperature also exhibited a very wide range, from 85-94°C with the sublittoral type generally melting at higher temperatures than the intertidal type. It has also shown the widest range in gel strength, from $<60$ to 240gm/cm$^2$.

It is worth noting here that the plants that grow as tufts on rocky surfaces generally had lower agar yields but of higher gel strength than the sublittoral or the ones that grow in pools. The low yield could be explained by the fact that there is lower productivity in the tuft form of seaweeds as compared to the sublittoral solitary forms since more energy

is expended by the former to survive the harsher physiological conditions (Taylor and Hay, 1984). The higher gel strength could also be a physiological adaptation. The tuft forms have to withstand a lot of physiological and physical stress due to desiccation during low tides and currents respectively, hence developing tougher cell walls.

Apart from the fact that G. salicornia type growing in shallow pools produced the lowest agar yield, they also had the lowest gel strength when compared to the other types. An explanation for this could be based on the fact that since they grow in pools they are not exposed to conditions as rough as those growing exposed on rocky surfaces and hence have not developed the tough cell walls. Furthermore due to the nature of their microhabitats the pool water temperatures can go as high as 36°C during low tide and these high temperatures have been reported to result in very poor quality agar (Craigie and Wen, 1984).

Agar from alkali-treated plants was of a lower yield than the native and the gelling temperatures were not significantly different. The gel strength, on the other hand, was significantly higher, though the difference was not very big (218gm/cm$^2$ as compared to 180gm/cm$^2$). Treatment of the native agar with alkali did not improve the gel strength

significantly.

G. verrucosa.

Native agar yield from this species was the highest, 29-30% dry weight though it was only possible to do the extraction during July when it was available in large enough quantities. Its paucity during the rest of the year therefore, made it not possible to study its agar content and quality during any other part of the year. The gel strength recorded for July was quite high 205-220gm/cm$^2$. However, its gelling temperature was one of the lowest as compared to the other species studied. It ranged from 28-29°C though the melting temperatures were high, 90-91°C. Table 7.

The yield of agar from alkali-treated plants was very low (9.1%) compared to the native of 30.3%, although it gave the best response in terms of gel strength (306gm/cm$^2$ vs the native 220gm/cm$^2$). The gelling temperature did not change significantly. Alkali-treated agar, on the other hand, showed no significant improvement on the gel strength.

Gracilaria sp.

Native agar yield from the species ranged between 10 and 16.8%

dry weight. Analysis of its native agar gave the highest gelling temperatures, ranging between 39 and 40°C and gel strength ranging from 145-203gm/cm$^2$. Its melting temperatures varied from 89-91°C. Seasonality cannot be conclusively discussed here because of the limited period of collection. However, from the work done there is a trend of better agar yield and quality during the month of November as compared to the other two months. This is the only species that showed quite a significant improvement on the gel strength of its alkali-treated agar (native 203gm/cm$^2$ and alkali-treated 278gm/cm$^2$). Table 8.

Table 3
Yield of native agar, gelling temperature and gel strength from G. corticata

| Station and date of harvest | Yield (%dry wt) | Gel Temp(°C) | GS gm/cm$^2$ | Melt. Temp(°C) |
|---|---|---|---|---|
| McKenzie | | | | |
| 18-10-89 | 18.1 | 33.7 ± 04 | < 60 | 82.0 ± 0.0 |
| 13-12-89* | 17.7 | 30.0 ± 0. | < 60 | 86.8 ± 0.8 |
| 25-07-90 | 8.8 | 33.7 ± 0.4 | < 60 | 84.5 ± 0.4 |
| 13-09-90 | 20.5 | 33.3 ± 0.5 | < 60 | 83.8 ± 0.3 |
| 24-10-90 | 19.1 | 34.9 ± 0.6 | < 60 | 86.0 ± 0.5 |
| 13-12-90* | 14.8 | 29.3 ± 0.4 | < 60 | 87.3 ± 0.3 |
| 18-01-91 | 13.8 | 32.0 ± 0.5 | < 60 | 85.5 ± 0.5 |
| 04-02-91 | 15.7 | 31.8 ± 0.3 | < 60 | 83.5 ± 0.8 |
| Gazi | | | | |
| 02-11-89 | 17.7 | 33.7 ± 1.0 | < 60 | 79.8 ± 0.8 |
| 17-01-90 | 10.1 | 33.7 ± 0.3 | < 60 | 87.8 ± 0.8 |
| 26-03-90 | 10.2 | 33.9 ± 0.3 | < 60 | 87.5 ± 0.0 |
| 26-04-90* | 10.7 | 29.8 ± 0.3 | < 60 | 90.5 ± 1.0 |
| 23-08-90 | 10.4 | 31.1 ± 0.4 | < 60 | 88.5 ± 0.3 |
| Mambrui | | | | |
| 30-11-89 | 26.7 | 35.4 ± 0.5 | 70 ± 4.0 | 85.5 ± 0.5 |
| 25-05-90 | 16.4 | 33.7 ± 0.4 | 80 ± 0.0 | 86.3 ± 0.3 |
| 20-03-91 | 15.3 | 33.5 ± 0.1 | < 60 | 88.5 ± 0.5 |
| Difcto Bacto Agar | - | 29.5 ± 0.5 | 373.3 ± 14.3 | 88.5 ± 0.3 |

* Cystocarpic plants

Table 4
Yield of native agar, gelling temperature and gel strength from G. crassa.

| Station and date of harvest | Yield (% dry wt) | Gel Temp(°C) | GS gm/cm$^2$ | Melt. Temp(°C) |
|---|---|---|---|---|
| Fort Jesus | | | | |
| 23-09-89 | 23.0 | 36.8 ± 0.5 | 195 ± 10.4 | 94.8 ± 0.8 |
| 16-10-89* | 24.0 | 36.2 ± 0.5 | 135 ± 14.7 | 91.0 ± 0.0 |
| 14-02-90 | 16.7 | 36.6 ± 0.4 | 125 ± 14.7 | 91.5 ± 0.5 |
| 25-06-90 | 17.0 | 36.3 ± 0.3 | 165.7 ± 7.1 | 93.8 ± 0.3 |
| 20-08-90 | 12.7 | 37.2 ± 0.3 | 205 ± 4.1 | 94.5 ± 0.5 |
| 24-10-90 | 25.3 | 37.2 ± 0.4 | 183.3 ± 13.1 | 91.8 ± 0.3 |
| Difco Bacto Agar | - | 29.5 ± 0.5 | 373.3 ± 14.3 | 88.5 ± 0.3 |

* Exposed at extreme low water spring

Table 5
Yield of native agar, gelling temperature and gel strength from G. millardetii.

| Station and date of harvest | Yield (% dry wt) | Gel Temp(°C) | GS gm/cm$^2$ | Melt. Temp(°C) |
|---|---|---|---|---|
| Fort Jesus | | | | |
| 15-09-89 | 12.6 | 33.0±0.2 | 68.3±2 | 87.5±0.5 |
| 16-10-89 | 17.2 | 32.0±0.6 | 75.3±5.1 | 86.3±0.3 |
| 24-07-90 | 8.7 | 34.5±0.0 | 99.0±4.1 | 92.5±0.3 |
| 24-09-90 | 11.9 | 30.7±0.4 | <60 | 91.8±0.3 |
| 24-10-90 | 16.2 | 33.0±0.4 | 73.3±2.3 | 90.3±0.8 |
| Difco Bacto Agar | - | 29.5±0.5 | 373.3±14.3 | 88.5±0.3 |

Table 6
Yield of native agar, gelling temperature and gel strength from G. salicornia

| Station and date of harvest | Yield (% dry wt) | Gel Temp(°C) | GS gm/cm$^2$ | Melt. Temp(°C) |
|---|---|---|---|---|
| McKenzie (pools) | | | | |
| 13-09-89 | 10.1 | 33.6±0.5 | 95.3±4.7 | 87.0±0.0 |
| 16-01-90 | 13.0 | 35.9±0.7 | 72.0±4.1 | 86.8±0.8 |
| 15-03-90 | 12.0 | 37.2±0.5 | 103.3±6.0 | 89.5±0.5 |
| 21-06-90 | 10.2 | 37.5±0.4 | 138.7±4.7 | 85.0±0.0 |
| 21-08-90 | 10.1 | 37.8±0.6 | 78.7±6.2 | 86.5±0.5 |
| 26-09-90 | 9.2 | 34.9±0.3 | <60 | 85.3±0.3 |
| McKenzie (rocky) | | | | |
| 21-06-90 | 8.1 | 38.3±0.2 | 240.0±8.2 | 88.8±0.3 |
| 21-08-90 | 8.1 | 37.8±0.3 | 160.3±2.4 | 87.0±0.0 |
| Gazi | | | | |
| 02-11-89 | 13.1 | 36.6±0.8 | 183.3±13.1 | 94.5±0.5 |
| 17-01-90 | 15.1 | 38.8±0.4 | 198.3±6.2 | 89.3±0.8 |
| 26-03-90 | 15.1 | 33.7±0.5 | 138.3±2.4 | 86.0±1.0 |
| 26-07-90 | 12.9 | 34.7±0.4 | 151.7±8.5 | 90.3±0.3 |
| 25-09-90 | 11.2 | 34.4±0.7 | 160.0±10.8 | 90.0±0.0 |
| 26-10-90 | 12.9 | 36.5±0.2 | 161.6±8.5 | 91.3±0.3 |
| 23-11-90 | 12.9 | 35.5±0.7 | 141.7±8.5 | 93.8±0.8 |
| Shelly Beach | | | | |
| 12-4-89 | 12.3 | 36.1±0.2 | 157.3±6.2 | 88.8±0.5 |
| 30-10-89 | 16.2 | 37.7±0.4 | 180.1±8.2 | 87.5±0.5 |
| Difco Bacto Agar | - | 29.5±0.5 | 373.3±14.3 | 88.5±0.3 |

Table 7

Yield of native agar, gelling temperature and gel strength from G. verrucosa

| Station and date of harvest | Yield (% dry wt) | Gel Temp(°C) | GS gm/cm² | Melt. Temp(°C) |
|---|---|---|---|---|
| Vanga 17-7-91 | 30.3 | 28.9 ± 0.2 | 220 ± 10.8 | 90.0 ± 0 |
| Gazi 20-7-90 | 29.1 | 29.5 ± 0.4 | 205 ± 9.2 | 91.3 ± 0.8 |
| Difco Bacto Agar | - | 29.5 ± 0.5 | 373.3 ± 14.3 | 88.5 ± 0.3 |

Table 8
Yield of native agar, gelling temperature and gel strength from Graçilaria sp.

| Station and date of harvest | Yield (% dry wt) | Gel Temp(°C) | GS gm/cm² | Melt. Temp(°C) |
|---|---|---|---|---|
| Mambrui | | | | |
| 30-11-89 | 16.8 | 40.4 ± 0.4 | 203.3 ± 6.2 | 91.5 ± 0.0 |
| 17-1-90 | 14.2 | 39.9 ± 0.5 | 199.4 ± 10.1 | 90.3 ± 0.3 |
| Mombasa Hosp. | | | | |
| 20-08-90 | 10.8 | 40.5 ± 0.3 | 143.0 ± 2.4 | 89.5 ± 0.5 |
| Difco Bacto Agar | - | 29.5 ± 0.5 | 373.3 ± 14.3 | 88.5 ± 0.3 |

### 7.1.1. Alkali Treatment and Gel Strength

Rees (1969) proposed that the gelling ability of the agar polysaccharides is due to the equatorial hydrogen atoms on the 3,6-anhydrogalactose residues which constrain the polysaccharide into a helix and the interaction of helices causes gel formation. Arnott et al. (1974) further did an X-ray analysis of agarose and indicated that individual molecules are arranged in a left-handed double helix with the strands parallel and three dimensional. They also reported that the helix is fully stabilised by interchain hydrogen bonds through the only unsubstituted positions O-6 and O-2 of the complementary galactose units.

It was also reported by Rees (1961; 1969) that helix formation is progressively inhibited by an increasing number of sulphate ester groups. Replacement of 3,6-anhydro-L-galactose with L-galactose sulphate causes kinks in the helix and hence a polysaccharide of lower gel strength is formed. If these sulphate groups are at C-6 they can be converted into 3,6-anhydro-L-galactose by treatment with alkali and an increase in gel strength results. Tagawa and Kojima (1972) went ahead and proved that the gelling ability of polysaccharides from G. verrucosa could be improved by alkali treatment which decreased ester sulphates. However,

Duckworth et al. (1971) showed that agars from G. ferox, G. damaecornis and G. domingensis improved very little following alkali treatment.

In the present study, when whole plants were alkali treated before extraction only G. verrucosa, G. crassa and G. salicornia yielded agar with significantly higher gel strengths, compared to the native, whereas G. corticata and G. millardetii did not improve much. Agar yield from alkali treated plants were all lower than native agar yield (Table 9). Durairatnam (1987) also reported a decrease in agar yield from alkali treated G. edulis and the gel strength was significantly improved after the treatment. In their study of G. chilensis from Chile, Matsuhiro and Urzúa (1990) also reported a decreased yield of agar when seaweeds are pretreated with alkali before extraction. They suggested that vigorous conditions in the procedure could cause some degradation of the polysaccharide.

With the exception of G. crassa and Gracilaria sp. alkali-treatment of agar extracts did not improve the gel strengths significantly (Tables 10 and 11). The chemical precursors for 3,6-anhydrogalactose, galactose-6-sulphate, which get converted to the sugar during alkali

treatment, hence increasing the gel strength, are known to form poor gels (Craigie and Wen, 1984). Since the samples in this study were hot water extracts only it is possible that most of these precursors were lost during the freeze-thawing stages. Furthermore, Lahaye et. al. (1986) showed that these chemical precursors had higher concentrations in the ethanol fractions than in the hot water fractions of the agar extracts. The fact that G. crassa and Gracilaria sp. extracts responded significantly to alkali-treatment shows that not all of their precursors for 3,6-anhydrogalactose was lost in the freeze-thawing process and this goes further to prove chemical heterogeneity there is even at the agaroid levels.

It would, therefore, appear that polysaccharides of G. verrucosa, G. crassa and G. salicornia contain agaroids which act as precursors for 3,6-anhydrogalactose sugar and on heating with alkali they give rise to anhydrosugars which consequently improve the gelling ability of the polysaccharide. On the other hand G. corticata and G. millardetii do not seem to pocess these precursors. May be their sulphate groups which are hydrolysed into methyl groups to form the 3,6-anhydro-L-galactose are not located on C-6. This can only be elucidated by a study of the structure of the polysaccharides which is beyond the scope of this study.

Duckworth et al. (1971) proposed that the resistance of the sulphate groups of the agars from G. ferox, G. damaecornis and G. domingensis on treatment with alkali indicates that most of the sulphate is not located at C-6. Testing further, they concluded that it is the arrangement of sulphate groups rather than the extent of 6-O-methylation, which prevents the G. foliifera agar from forming a firmer gel. It may, therefore, be concluded that the extent to which alkali treatment improves the gel strength of agar varies from species to species.

Table 9
A comparison of yield, gelling temperature and gel strength of agar from plants treated with and without alkali before extraction.

| Species | Yield (%dry wt) | Gel Temp(°C) | GS gm/cm² |
|---|---|---|---|
| G. corticata | | | |
| Mambrui | | | |
| Native | 15.3 | 33.5 ± 0.1 | < 60 |
| Hydrolysed | 8.9 | 32.6 ± 0.2 | < 60 |
| McKenzie | | | |
| Native | 13.8 | 32.0 ± 0.5 | < 60 |
| Hydrolysed | 6.0 | 33.3 ± 0.6 | < 60 |
| G. crassa | | | |
| Native | 16.7 | 36.6 ± 0.4 | 125.0 ± 14.7 |
| Hydrolysed | 12.2 | 36.2 ± 0.3 | 198.3 ± 10.7 |
| G. millardertii | | | |
| Native | 8.7 | 34.5 ± 0.0 | 99.0 ± 4.1 |
| Hydrolysed | 4.5 | 33.8 ± 0.6 | 107 ± 10.2 |
| G. salicornia | | | |
| Native | 16.2 | 37.7 ± 0.4 | 180.0 ± 8.2 |
| Hydrolysed | 8.5 | 37.1 ± 0.6 | 218.1 ± 10.1 |
| G. verrucosa | | | |
| Native | 30.3 | 28.9 ± 0.2 | 220 ± 10.8 |
| Hydrolysed | 9.1 | 28.9 ± 0.1 | 306 ± 7.0 |

Table 10
Gel strength 3,6-anhydrogalactose and sulphate content of native agar from different species.

| Species and date of harvest | | GS ($gm/cm^2$) | 3,6-anhydro (%) | Sulphate ($SO_4^{-2}$ %) |
|---|---|---|---|---|
| G. corticata | | | | |
| McKenzie | 13-09-90 | < 60 | 14.5 | 2.8 |
| Gazi | 02-11-89 | < 60 | 18.7 | 3.05 |
| Mambrui | 25-05-90 | 80 | 18.9 | - |
| Mambrui | 30-11-89 | 70 ± 4 | 15.8 | 2.65 |
| G. crassa | | | | |
| | 20-08 90 | 205.0 ± 4.1 | 21.4 | 2.0 |
| | 14-02-90 | 125 ± 14.7 | 16.9 | 2.2 |
| G. millardetii | | | | |
| | 24-07-90 | 99.0 ± 4.1 | 19.2 | 1.9 |
| | 24-09-90 | < 60 | 15.2 | 2.1 |
| G. salicornia | | | | |
| McKenzie(p) | 21-06-90 | 138.7 ± 4.7 | 11.1 | 1.25 |
| McKenzie(r) | 21-06-90 | 240.0 ± 8.2 | 19.1 | 1.6 |
| Shelly Bc | 30-10-89 | 180.1 ± 8.2 | 18.6 | 1.45 |
| Gazi | 17-01-90 | 198.3 ± 6.2 | 19.4 | 1.45 |
| G. verrucosa | | | | |
| | 17-07-91 | 220.0 ± 10.8 | 23.0 | 2.3 |
| Gracilaria sp. | | | | |
| Mambrui | 30-11-89 | 203.3 ± 6.2 | 20.5 | 1.3 |
| Difco Bacto Agar | | 373.3 ± 14.3 | 26.5 | - |

Table 11

Gel strength, 3,6-anhydrogalactose and sulphate content of alkali-treated agar from different species.

| Species and date of harvest | GS ($gm/cm^2$) | 3,6-anhydro (%) | Sulphate ($SO_4^{-2}$ %) |
|---|---|---|---|
| G. corticata | | | |
| McKenzie 13-09-90 | <60 | 16.9 | 2.6 |
| Gazi 02-11-89 | <60 | 21.2 | 2.7 |
| Mambrui 30-11-89 | 75 | 21.8 | 2.6 |
| G. crassa | | | |
| 14-02-90 | 155 | 18.2 | 1.9 |
| G. millardetii | | | |
| 24-09-90 | <60 | 21.2 | 2.0 |
| G. salicornia | | | |
| Gazi 17-01-90 | 210 | 20.9 | 1.15 |
| G. verrucosa | | | |
| 17-07-91 | 215 | 20.7 | 1.65 |
| Gracilaria sp. | | | |
| Mambrui 30-11-89 | 278 | 22.3 | 0.85 |

### 7.1.2. Gelling and Melting Temperatures

The gelling and melting temperatures are important physical properties of agar which mark the transition from the sol to the gel state and from the gel to the sol state respectively. The gelling temperature recorded in this study is the dynamic gelling temperature as opposed to the isothermal gelling temperature which can be defined as the lowest temperature at which a 1.5% solution of agar when left for an indefinite period of time will not gel. The mechanism of gel-sol transition is illustrated by reference to the model system proposed by Rees (1969), figure 40. In the sol state all the solute molecules have sufficient kinetic energy to keep them as random coils, but as the solution cools double helices begin to form (Gel 1), and with further cooling the double helices interact to form aggregates and hence a firm gel is formed (Gel 2). This is a thermoreversible transformation. On heating the interaction between the double helices become weak and hence the gel become disorderly and strands disperse into random coil form (Letherby and Young, 1981).

Bird et al. (1981) observed a high positive correlation between gel strength and melting temperature, which is also true of the present study ($r = 0.613$). Selby and Wynne (1973) suggested that the two properties of agar are an indicator of molecular weight and size of the agar

polymers. The longer polymers may be more capable of interacting with each other and forming a greater three dimensional lattice between water and the gel helices, resulting in agars with higher gel strengths and melting temperatures. The gelling temperatures are indicators of the extent of methylation in agar extracts such that high methylation goes hand in hand with high gelling temperatures (Guiseley, 1970). On this score one would therefore infer that of the agar extracts studied that of G. verrucosa has the lowest methoxyl content while the one from Gracilaria sp. has the highest.

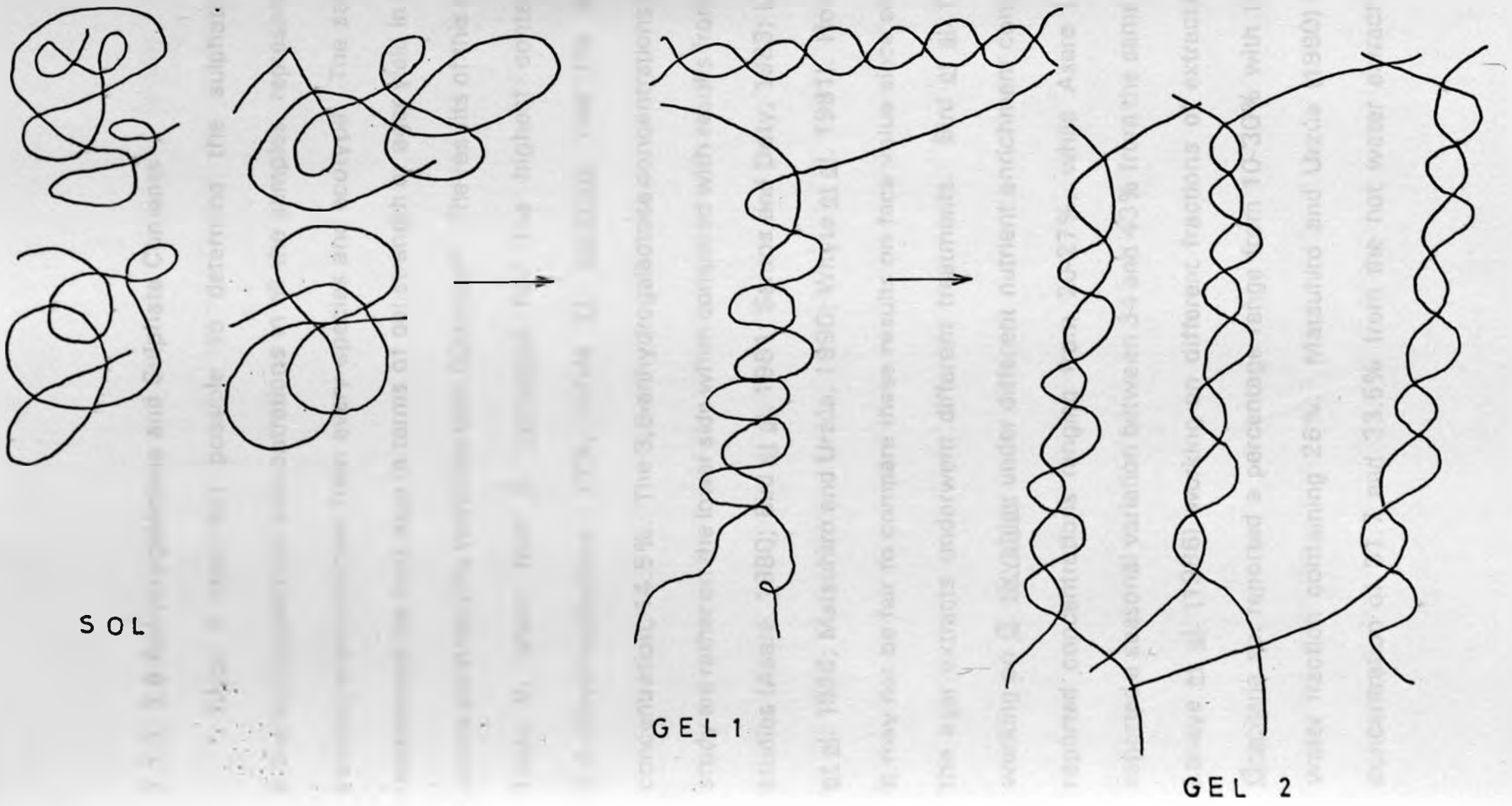



Figure 40 Gel - Sol Transition model for agar (after Rees, 1969)

### 7.1.3. 3,6-Anhydrogalactose and Sulphate Contents

Since it was not possible to determine the sulphate and 3,6-anhydrogalactose concentrations in all the samples, representative samples were selected from every species and ecotype. The samples represented the best agar in terms of gel strength or agar yield in cases where gel strengths were less than 60gm/cm$^2$. The results of this study, Table 9, show that G. verrucosa had the highest content of 3,6-anhydrogalactose, 23%, while G. corticata had the lowest concentration, 14.5%. The 3,6-anhydrogalactose concentrations in this study are rather on the lower side when compared with results from other studies (Asare, 1980; Bird et al. 1981; Santos and Doty, 1983; Lahaye et al. 1986; Matsuhiro and Urzúa, 1990; Whyte et al. 1981). However, it may not be fair to compare these results on face value since some of the agar extracts underwent different treatments. Bird et al. (1981) working on G. tikvahiae under different nutrient enrichment conditions reported concentrations ranging from 20-27%, while Asare (1980) reported a seasonal variation between 34 and 43% from the same plant. Lahaye et al. (1986) working on different fractions of extracts from Gracilaria sp. reported a percentage range from 10-30% with the hot water fraction containing 26%. Matsuhiro and Urzúa (1990) had a concentration of 31.7 and 33.5% from the hot water extract of G.

chilensis from two different stations while Doty and Santos (1983) working on G. cylindrica observed a range of 29-42% depending on date of collection. Whyte et al. (1981) had a seasonal variation of 20-35% from G. verrucosa.

3,6-anhydrogalactose content in the present study showed variations depending on species, ecotype and time of collection. It is a general principle that the higher the concentration of the sugar in agar extract the firmer the gel (Asare, 1980; Whyte et al. 1981). However, the present results show that the amount of the sugar needed to produce a given gel strength varies from species to species and from one ecotype to the other. For instance, a concentration of 18.6% of the sugar in G. salicornia gave a gel strength of over 180gm/cm$^2$ whereas similar concentrations in G. corticata yielded gel strengths of 80gm/cm$^2$ and below; 19% concentration of the sugar in G. salicornia, the type that grows as cushions on rocky surfaces, gave 240gm/cm$^2$ gel strength whereas the same concentration for the sublittoral type had a gel strength of 198gm/cm$^2$.

The sulphate content also varied depending on species and ecotypes. It was highest in G. corticata and lowest in Gracilaria sp. The

high concentration in G. corticata is consistent with the fact that its gel strength was the lowest. However, the relatively high concentration in G. verrucosa. in comparison to the other species is inconsistent with the fact that the species had one of the best gel strengths and the highest 3,6-anhydrogalactose content. Looking at the results from the alkali-treated agar of the same species it is apparent that the concentration of both the sugar and sulphate decreased. Asare (1981) observed a positive correlation between sulphate content and 3,6-anhydrogalactose concentrations in Neoagardhiella baileyi, and he attributed it to the fact that some of the sulphate groups were attached to the anhydrogalactose unit thus an increase in the sugar resulted in an increase in the sulphate, which should be the other way round (Rees, 1961; 1969). This could be the case in G. verrucosa where reduction in the sugar and sulphate contents occurred at the same time.

Lahaye et al. (1986) reported that the concentration of alkali-labile sulphate, obtained by subtraction of the sulphate values obtained before and after alkali treatment of the polymers, was higher in the ethanol extracts than in the hot water extracts from two different species, G. tenuistipitata and G. blodgettii. But in the case of G. eucheumoides agar, they reported a consistently higher alkali-stable sulphate in all the

fractions including the ethanol extracts. These results would partly explain the lack of substantial decrease in the sulphate content of the extracts that were alkali treated in the current study since they were hot water extracts. Furthermore, it is possible that the alkali-labile sulphate had been lost during the freeze-thawing process as they are known to be soluble in cold water (Miller and Furneaux,1987; Yaphe, 1984). Lack of response to alkali treatment by G. corticata and G. millardetii at either plant or polymer level could also be attributed to high concentration of alkali-stable sulphate, since the greater the amount of alkali-stable sulphate the poorer the gel strength. On the whole sulphate concentrations of the species studied is generally high compared to the concentrations reported from the temperate waters. This is in conformity with observations made by Craigie and Wen (1984) which showed that plants grown at high temperatures had higher sulphate concentrations than those grown at lower temperature. It should be noted here that the water temperatures under which the present samples were found growing ranged between $27^0$ and $32^0$ C and went as high as $36^0$C during low tides.

**7.1.4 Categorization of Agar gels on basis of substituent groups:**

Yaphe (1984) divided Gracilaria agars into 3 groups depending on

the substituted groups on the agarose.

Group 1 Those that exhibit a high gel strength and is basically composed of neutral agarose molecules. This group is further divided into two subgroups;-

(a) agarose with low methoxyl content, indicated by low gelling temperatures and

(b) agarose with high methoxyl content and D-Galactose residues replaced by 6 - 0 - methyl - D - galactose.

Group 2 Those that exhibit gel strength that is lower than those in group 1 and having a major fraction of agarose molecules substituted with sulphate and pyruvate groups.

Group 3 These agars show a low gel strength and their charge density is attributed to sulphate.

According to this scheme of grouping it is possible to categorize the Gracilaria agars analyzed in the present study as follows:-

Group 1(a) G. verrucosa since it had the highest recorded gel strength and lowest gelling temperature indicative of low methoxyl

content.

1(b) G. crassa and Gracilaria sp. with high gel strength and high gelling temperatures indicative of high methoxyl content.

Group 2 G. salicornia with generally lower gel strength than those in group 1 and with relatively less improvement of gel strength on alkali treatment, indicative of a greater proportion of alkali stable sulphates.

Group 3 G. corticata and G. millardetii with very low gel strengths that hardly change on alkali treatment, indicative of very high proportions of alkali-stable sulphate.

## 7.2. A comparison of agar extracts from different species with commercial agar

1.5% solution of Difco Bacto Agar, manufactured by Difco Laboratories, U.S.A. was prepared in the same way as the extracts and its gel strength and gelling temperature measured using the same apparatus. The gelling temperature was 29.5°C, the melting temperature 88.5°C and the gel strength 373.3gm/cm$^2$. In comparing results obtained from native agar from different species and the commercial agar it is

clear that G. verrucosa, G. crassa, G. salicornia, sublittoral type, and Gracilaria sp. have the closest characteristic in terms of gel strength, which is the most important property. G. millardetii and G. corticata on the other hand are the poorest. However, in terms of gelling temperature all the species except G. verrucosa had significantly higher temperatures.

A comparison of gel strengths, gelling and melting temperatures from the present study with the results of other reports may not be very useful since different gel testers have been used. Hoyle (1975) suggested that the use of different sizes of plunger may account for the varying results obtained between one sample and the other. Diameters of plungers have been known to range from 8mm (Duckworth et al. 1971) to 1.0945cm (Santos and Doty, 1978). In the light of these variations, a comparison between samples and a commercial product using the same apparatus would therefore be more appropriate.

### 7.3. Agar Yield and Seasonality

From the above results it is clear that Gracilaria species studied during the period between September and November gave the highest agar yield with corresponding better quality in terms of gel strength. However, this generalisation is not true for G. salicornia which has not

shown any specific pattern of seasonality both in agar yield and quality. The diversity in types of this species on the Kenyan coast calls for a longer period of study before any pattern can be conclusively reported. It is important to note here that the period mentioned above as the peak for agar yield for the different species coincides with most of the species maximum biomass period (Figure 25). G. verrucosa, however, had its peak biomass and agar yield in July. On the other hand, the agar content of the various species was relatively low during the period of regeneration for most of the species. Similar results have been reported by Oza (1978); Hoyle (1978a); Bird et al. (1981). This could be explained by the fact that during regeneration there is a greater propotion of younger tissues which according to Craigie and Wen (1984) contain more of the chemical precursors for the 3,6-anhydrogalactose than the older tissues and less of the highly substituted agar. Since these precursors have poorer gelling properties they could have been lost during the freeze-thawing procedure. Cold water extracts would have given the correct picture, but unfortunately this was not possible technically.

Seasonal changes have been observed in several chemical components, including agar, of different Gracilaria species (Humm, 1951; Kim and Humm, 1965; Umamaheswara, 1969; John and Asare, 1975;

Penniman, 1977; Hoyle, 1978b). De Loach et al. (1946) reported that G. confervoides in North Carolina exhibited the highest yield of agar in August, while its peak biomass occurred in October. Agar in G. foliifera was reported to have declined in autumn as the algae reached its maximum abundance (Humm, 1951). On the other hand, the abundance and agar content peaked simultaneously in G. dentata from Ghana (John and Asare, 1975) and in G. corticata from India (Umamaheswara, 1969). Current study on G. corticata depict similar findings.

Random seasonal fluctuations in agar of G. bursapastoris from Hawaii provided no definite seasonal trends whereas G. coropifolia exhibited a definite decline in agar content to its lowest level in February, a time at which biomass of both species was at its maximum (Hoyle, 1978a). Similar random seasonal fluctuations in agar content has been observed for G. salicornia in the current study. Correlation between biomass and agar yield would therefore appear to be species dependent, though in the present study the majority of the species had simultaneous biomass and agar peaks.

In his study of G. corticata from Veraval, India, Oza (1978) reported that there was no significant seasonal variation in its gel

strength which showed a narrow variation from 15-17gm/cm$^2$. However, he reported a significant difference in seasonal, yields, with the lowest yield of 14.5% recorded in the months of August to October and May while the highest yield ranging from 22.0 to 22.5% was obtained in the months of June - July and November to April. These figures are quite comparable to those reported in the present study. The variation in the gel strength of the same species in this study has not been revealed since most of the readings were below 60gm/cm$^2$. However, it is clear that the species had the lowest gel strength when compared to the other species. On the contrary, variations in gel strength for the other species, namely, G. crassa, G. millardetii and Gracilaria sp. tended to show seasonal peaks. The best gel strength for G. crassa and Gracilaria sp. coincided with their maximum agar yield. G. millardetii, on the other hand yielded agar of better strength during its low agar yield period. Random fluctuations in the gel strength of G. salicornia did not show any definite seasonal trend. The relationship between agar yield and gel quality therefore, varies from species to species.

# CHAPTER 8

# CONCLUSION

Kenya's most actively utilised marine resources are fisheries and mangrove forests (Kokwaro, 1985; Nzioka, 1985). Utilization of seaweed as a resource has been quite passive and this may be attributed to lack of knowledge on the potential there is in these plants. This study has endeavoured to throw some light into a tiny portion of the Kenyan seaweed, the genus Gracilaria, indicating the composition and distribution of the taxa along the coast, factors governing its distribution and the properties of its commercially sought after polysaccharide, agar.

Morphological diversity of the genus Gracilaria has been cited as a major deterrent in the identification of members of the genus. These taxonomic problems are currently both immediate and serious. It has been difficult, frequently impossible, to relate various results and information to a particular species with certainty. The search for a breakthrough into these taxonomic problems is on worldwide and it would be in order to state that the current study has been part of this search. Present results have shown that a taxonomic study based on morphology, when coupled with ecological information pertaining to

individual specimens can quite reliably be used to identify the Kenyan species. Most botanists have listed Kenyan Gracilaria species without drawing up a systematic identification procedure, which the current study has come up with.

Members of the genus have become important sources of raw material for the agar industry (Santos, 1980). With a high demand for agar (Moss, 1978) and decreasing harvests of the excellent agarophyte Gelidium (Duckworth et al., 1971), various Gracilaria species have been increasingly utilised for their agar product and the global search for the best species and strain is actively going on. Industrial applications are dominated by three quality grades of agar (Santelices and Doty, 1989):

(a) **Sugar reactive agar** whose gels are consistently stronger as a function of sugar concentrations

(b) **"Standard agar"** having the temperature and other requirements for microbiological purposes and

(c) **Food-grade agar** which is any agar not meeting the standards for either (a) or (b).

The large scale users of agar are concerned with the physical and

chemical functioning of the agar as a raw material in a particular application. Gracilaria is now considered to be the major raw material for the production of food and industrial agar (Doty and Santos, 1983; Yaphe and Duckworth, 1972) mainly because their agar form gels that are soft and elastic. International trade in dried Gracilaria is based largely on gel strength, the higher the gel strength the higher the price paid.

The results of this study have demonstrated that different species of Gracilaria provide considerable variations in yield, physical and chemical qualities of the constituent agar. They have further shown that these variations also occur within species depending on the prevailing ecological conditions and also on season. Considering gel strength which is the most important agar quality, the strain of G. salicornia that grows as cushions on rocky surfaces yields the best native agar (gel strength 240gm/cm$^2$) followed by G. verrucosa (gel strength 220gm/cm$^2$). However, upon alkali treatment , which is one of the industrial treatments for agar production, G. verrucosa is superior to G. salicornia since this treatment resulted in raising the gel strength by 86gm/cm$^2$ in the former and in the latter the gel strength was raised by 38gm/cm$^2$. This makes the gel strength of alkali treated G. verrucosa (306gm/cm$^2$) be the closest to that of the commercial agar tested (373gm/cm$^2$). It is not only the

quality of agar that is important but the quantity must also be taken into consideration. In terms of native agar yield G. verrucosa ranks top of the species tested with a maximum yield of 30% while G. salicornia ranks least with a maximum yield of 16.2%. Overally, therefore, G. verrucosa is the best of the species tested for agar production. The second best species in terms of native agar yield and gel strength is G. crassa, with a maximum agar yield of 25% and gel strength of 205gm/cm$^2$. It also showed quite a significant increase in the gel strength (73gm/cm$^2$) after alkali treatment. Gracilaria sp yielded agar of reasonably firm gel (max. gel strength 203gm/cm$^2$) but its agar yield is quite low, maximum 16.8%. G. corticata and G. millardetii have fallen far short of the commercial standard both by virtue of their agar yield and the strength of their gels.

Based on the substituent groups on agarose the Gracilaria agars studied can be divided into three major groups:-

Group 1 (a) G. verrucosa with a high gel strength and low gelling temperature, indicating high concentrations of 3,6-anhydrogalactose and low methoxyl content

1 (b) G. crassa and Gracilaria sp. with high gel strength and high gelling temperatures indicating high levels of

both 3, 6- anhydrogalactose and methoxyl groups.

Group 2. G. salicornia with a higher proportion of alkali- stable sulphate than in group 1 and lower levels of 3,6-anhydrogalactose thus resulting in lower gel strengths.

Group 3. G. corticata and G. millardetii with the highest level of alkali- stable sulphate and low contents of 3,6-anhydrogalactose thus resulting in the lowest gel strength.

The United States Pharmacopoeia standards require that agars have a gelling temperature between 32-39°C and that they do not melt below 85°C (Hurtado-Ponce and Umezaki, 1988). Agar from G. crassa has gelling temperature varying between 36 and 37°C and melting temperature ranging from 91 to 94°C which fall within the above requirements. G. verrucosa, on the other hand, produces agar whose gelling temperature varies between 28 and 29°C and melting temperature varying between 90 and 91°C. According to the present results the gelling temperature for one of the United States commercial agar, Difco Bacto Agar, is 29 ± 5°C and it melts at 88.5°C. This is one of the commonly used "standard agar". Humm and Williams (1948) reported

a 1.5% solution of the same commercial agar as having a gel strength of 250gm/cm$^2$, gelling temperature of 34°C and a melting temperature of 70°C. Differences between their results and those of the present study could be attributed to the use of different instruments. Gelidium, which is one of the excellent sources of agar, has been reported to have a gel strength of approximately 300gm/cm$^2$ (Duckworth et. al., 1971).

Observations made in the current study would further reinforce conclusions made by Duckworth and Yaphe, 1971; Duckworth et al. 1971; and Lahaye et al. 1986, that chemical heterogeneity of agar synthesized by Gracilaria species is complex. The concentration and distribution of substituent groups on agar and hence the physical and chemical properties of the polymers vary according to the species, physiological and ecological conditions under which the plants have grown. Prior knowledge of the type of variations existing within species, which this study has endeavoured to establish, is especially important when considering the algae for commercial production of agar. For this knowledge to be consistent and useful a clear identification system, which the current study has also established for the Kenyan taxa, is vital. Due to lack of consistent taxonomical information on the genus agar producing industries have tended to develop their own system of

classification based on certain characteristics that are useful to them (Stancioff, 1981). However, for the producer of the plants as industrial raw material it is paramount that he is able to classify his plants with consistency.

### 8.1. Agar Importation In Kenya

In the course of this study it became imperative to carry out a survey of the domestic market for agar. The survey was meant to establish the different types of agar being imported for use locally, their value, who the consumers are and their country of origin. Annual Trade Reports (1980-1990) were obtained from the Customs and Excise department, Ministry of Finance. It is unfortunate that statistics for agar imports up to 1989 had been lumped up together with "other materials of vegetable origin" under the SITC code 292919. The report for 1990, however, had statistics for agar import under e separate code, 29296100 though the entries did not seem to be complete. The new code for agar importation had been effected by 1989 though the Annual Trade Reports have not reflected this. A report from the Central Bank of Kenya (Personal communication), showed that in 1989 an allocation of Ksh. 1,250,801 was made for the importation of agar-agar under the new code. They, however, did not have any records for 1990 by the time this report was

obtained. An attempt to get information directly from importers, whose contacts were obtained from the customs department, was not very fruitful as most of them did not want to reveal information. Some of them did respond positively.

Looking at the data compiled from these reports, Table 12, one gets a rough idea as to how much is being imported into the country, though it may not give the exact figures due to the limitations mentioned above. In addition to that, importers do not import the product on yearly basis so long as they have enough in stock. Now that there is a separate entry code for agar, importation statistics for the next decade will give a better picture on its importation into the country. From 1990 list of importers it is evident that the bulk of agar imported during the year was "standard agar", which is mainly used by Universities, research institutions, schools and hospitals and most of it originated from the United Kingdom. These figures give an impression that the domestic market for agar is not large enough to warrant a venture into the industry. Nonetheless, international market for the product is not saturated (Santelices and Doty, 1989) so long as a product that meets its standard can be produced. Furthermore, Kenya is a developing country and the demand for the product is bound to increase.

Table 12

Statistics showing importation of "Materials of Vegetable Origin," including Agar-Agar, for the last 10 years.

| Year of import | Import. code | Weight (Kg) | Value of import Ksh.(without duty) |
|---|---|---|---|
| 1980 | 292919 | 34,869 | 1,785.428 |
| 1981 | ,, | 19,517 | 1,341,172 |
| 1982 | ,, | 17,316 | 1,704,187 |
| 1983 | ,, | 23,144 | 2,180,695 |
| 1984 | ,, | 19,155 | 2,088,295 |
| 1985 | ,, | 17,338 | 1,173,314 |
| 1986 | ,, | 43,071 | 3,339,126 |
| 1987 | ,, | 177,543 | 3,504,056 |
| 1988 | ,, | 14,715 | 2,149,522 |
| 1989* | ,, | 787 | 84,022 |
| 1990* | 29296100 | 342 | 315,507 |

Data obtained from Annual Trade Reports, Customs and Excise Department, Ministry of Finance.

*Data not complete

## 8.2. Need for Phycoculture

From the results of these studies it has come to light that the coastal waters of Kenya do not have populations of Gracilaria that are large enough to support an industry. Even if there were, records from other countries (Santelices and Doty, 1989) have shown that an industry based on the wild population alone may not be stable. Strains of G. verrucosa are being successfully cultured in other countries (Bird and Ryther, 1990; Tseng, 1981) and through screening, strains that yield agar with high gel strengths ($>800 gm/cm^2$) are being isolated for culturing purposes (Levy et al. 1990). Santelices and Doty (1989) reported that all commercial forms of Gracilaria are of the slender and willowy nature with terete axes, usually less than 2mm in diameter. The strain of G. verrucosa reported in the current study just fits this description. Looking back at the results obtained from seasonality studies of this species, it appears to be actively growing only during a very short period of the year and hence one would be made to think that its cultivation throughout the year may not be a success. Destombe et al. (1988) have shown that despite its short period of good growth in the wild in most parts of the world it is possible to cultivate it throughout the year.

In the literature search made during this study, there is no mention of mass culture of G. crassa for agar production. This could be attributed to the fact that Gracilaria cultivation is actively being carried out in regions where there are other superior species, in terms of agar yield and quality, that could be considered for this. Since this species has been reported to be one of the best agarophytes in the Kenyan waters it would be commendable to develop it further by carrying out investigations that would enable its mass cultivation and possibly isolate better strains. G. crassa, unlike G. verrucosa, is typically tropical and it would be worthwhile having a go at it.

Kenya is blessed with several natural factors such as abundance in sunshine and the absence of destructive typhoons. Most of the coastal waters are relatively unpolluted apart from areas around major urban centres like Mombasa. The barrier reefs shelter inner lagoons from strong waves thus creating excellent sites for Gracilaria phycoculture, which requires sheltered waters (Tseng, 1981; McLachlan and Bird, 1986). Aquaculture in general has not been intensively developed and as such offers no strong competition to phycoculture. The political stability prevailing in the country together with its well developed infrastructure, good shipping and communication network pose few

problems for the marketing of the product. A larger proportion of the rural coastal population derives its livelihood from the sea and hence adopting seaweed farming may not be a problem if only they can be exposed to seaweed farming and its value, and this would widen their opportunities to earn a living.

Not withstanding the bright prospects, there exist a number of constraints and problems that need to be overcome if seaweed industry is to be developed. First and foremost is inadequacy of technical capability and expertise. There is lack of technology for seaweed culture in Kenya at the moment and it will take a deliberate, well designed concentrated effort to make it successful enough to compete with other seaweed producing countries. A constant supply of consistent quality is important if seaweed products from Kenya are to be competitive with those of other countries. In addition to technological constraints, the potential development of seaweed industry may be affected by market and economic constraints. While the domestic market may be able to support a small number of agar producing enterprises, the full scale development of the industry will very much depend on a reliable regional and international market. Considering the stiff competition that may be faced in the international market, good quality products will definately be

necessary.

On the whole the occurrence of ecotypic variation in seaweeds has been taken for evidence of genetic differentiation among populations whose members are perhaps in the process of speciation (Innes, 1984). It is on this proposition that efforts are directed at selection of strains with both morphologically and physiological desirable qualities. Studies have shown that morphological mutants of some Gracilaria species produce agar of higher quality than the wild type when grown under the same conditions (Levy et at. 1990; Patwary and Van de Meer, 1983).

Current study has clearly demonstrated species and ecotypic variations in the quantity and quality of agar extracts. The strain of G. verrucosa reported in the study has emerged as the best in terms of agar quality and quantity. Morphologically this strain also exhibits desirable characteristics for mass culture. Using the results of this study suitable sites for its culture could easily be identified. It could therefore be deduced that culturing this particular strain within suitable sites in the Kenyan waters could be viable. Growing of seaweeds for commercial production was not part of the aim of this study. However, from the results obtained it would be concluded that with further research on

suitable culture methods and strain selection of G. verrucosa, seaweed industry could be a reality in Kenya.

## GLOSSARY

| | |
|---|---|
| Apex: | Tip |
| Apical cell: | A prominent meristematic cell at the tip of a plant |
| Benthic: | Bottom-dwelling, attached to or resting on some substrate |
| Caespitose: | Growing in tufts or clumps |
| Cartilaginous: | Firm and elastic-like. |
| Carpogonium: | Female sex organ |
| Carposporangium: | The reproductive cell of the carposporophytic generation in the rhodophytes |
| Carpospore: | The diploid spore released from a carpogonium |
| Carposporophyte: | Diploid phase of life cycle of some red algae which develops within the female gametophyte |
| Compressed: | Flattened, usually laterally |
| Conceptacles: | Cavity within a thallus opening to the exterior through a pore or pores and containing reproductive organs |
| Confluent: | Merging into one |
| Cortex: | The outermost layer of cells or tissue in a thallus |

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| Cruciate: | Arranged in the form of a cross |
| Cystocarp: | Structure developed after fertilization |
| Decumbent: | Trailing on the ground |
| Dichotomous: | Having divisions always in pairs |
| Discoid: | Like a disc |
| Ecotype: | This is the product of a genotypic response of a species to a particular environmental condition of a habitat. |
| Epiphytic: | Plant attached to another plant, not growing parasitically upon it but merely using it for support |
| Eulittoral: | Near the shore, intertidal, littoral |
| Euryhaline: | Having a broad tolerance to varying salinities |
| Eurythermal: | Having a broad tolerance to varying temperatures |
| Gametophyte: | Phase of life cycle of plants which has haploid nuclei and during which sexual reproduction takes place |
| Gametangium: | A container in which gametes are produced |
| Gonimoblast: | A filament bearing a carpospore(s) or the entire collection of these filaments comprising the |

| | |
|---|---|
| | carposporophyte |
| Heterothallic: | Condition in which sexual reproduction occurs only through participation of two thalli |
| Holdfast: | An attaching cell or cells |
| Hypogynous: | Growing below the base of the female reproductive organ |
| Intercalary: | Inserted at a point a long a thallus, not terminal or basal. |
| Neustonic: | Living at the interface of water and the atmosphere |
| Ostiole: | Pores through which spores or gametes are discharged |
| Parenchymatous: | Tissue consisting of thin-walled cells with intercellular spaces containing air |
| Pericarp: | A sterile covering around the carposporophyte |
| Planktonic: | Living as a minute organism suspended in water |
| Proliferation: | Growth by active cell division |
| Prostrate: | Lying flat upon the substrate |
| Pseudodichotomous: | The paired branching pattern is false |
| Pustule: | Small areas of growth that are raised above |

| | |
|---|---|
| | the surface |
| Schizogenous: | Originating by separation of cells |
| Sori: | Group of sporangia |
| Spermatangium: | Male sex organ |
| Spermatium: | Non-motile male sex cell |
| Sporangium: | Organ within which asexual spores are produced |
| Sporophyte: | Phase of life-cycle of plants which has diploid nuclei and during which spores are produced |
| Sublittoral: | A depth below the lowest level of low tide |
| Succulent: | Having juicy tissues |
| Ramifications: | Side branches emanating from the main branch |
| Rhizoid: | An anchoring and / or absorptive organ lacking vascular tissue and a root cap |
| Terete: | Circular in transverse section |
| Tetraspore: | First cell of gametophyte generation formed in fours in a tetrasporangium following meiosis |
| Thallus: | Simple, vegetative, plant body, showing no differentiation into root, stem or leaf |

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