The development of *Mazzaella capensis* (J. Ag.) Fredericq in culture, in the field, and the effect of environmental factors.

By:

Nicola Claire Alexander

Honours Project
Department of Botany UCT
Faculty of Science
University of Cape Town

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ABSTRACT

Past research on *Mazzaella* species in Chile and Pacific North America concentrated on their carrageenan-producing potential and of patterns and mechanisms of gametophyte / sporophyte alternation. The South African Atlantic species, *Mazzaella capensis*, however has been neglected in these studies. This project sets out to investigate some basic hypotheses on the biology of *M. capensis.* The rate of development of *M. capensis* sporelings in culture was found to be faster at 18°C than at 15°C, this was thought to be related to the increased rate of reaction with increased temperature. The fact that *M. capensis* sporelings were able to tolerate temperatures of between 15 and 18°C is related to the biogeography of the organism and the temperature extant during their origin and dispersal. Growth rate of *M. capensis* sporelings was light saturated at 104μmol.m^{-2}.s^{-1} and is similar to *Gigartina polycarpa* and *Sarcothalia stirata.* Although this is a fairly high value for intertidal sporelings it is low considering the total sunlight received on a sunny day can be over 2000μmol.m^{-2}.s^{-1}, other studies have shown that adult seaweeds on the midshore have generally higher light saturation values (in the range of 150 - 300μmol.m^{-2}.s^{-1}). In the field there are many more factors such as nutrient availability, water movement and grazing and complex interactions, such as competition for space nutrients and light with other organisms, which influence development of the crust and the plant. Each individual crust-holdfast complex and its related fronds were found to be monophasic with respect to life history stage, this is thought to be a result of coalescence of crusts formed by spores released together by one parent. Within the *M. capensis* population at Kommetjie there was a switch of dominance from tetrasporophytes in early winter to gametophytes in early summer, corresponding with an increase in biomass. It

was concluded that the new recruits were mostly gametophytes and that their increase relative to the perenating tetrasporophytes was responsible for the switch in dominance. The coalescence of *M. capensis* crusts during developmental stages suggests that it is a clonal plant. It is similar to the clonal *M. cornucopiae* as it did not exhibit characteristics of self-thinning.

INTRODUCTION

Mazzaella capensis (J. Agardh) Fredericq, formerly *Iridaea capensis* J. Agardh (Hommersand *et al.*, 1994) (Gigartinales, Rhodophyta) has strap-like brown blades with the sori (dark spots) scattered over all but on the surface ? the most basal part of the blade (Branch *et al.*, 1994). The blades are cuneate and somewhat channeled near the base, and are broadly lanceolate in their general outline with an undulating margin (~~Anderson~~ Stegenga *et al.*, 1997). The thallus is multiaxial, with a filamentous medulla in the form of a wide meshed network of thin cylindrical cells. The cortex consists of bundles of anticlinal small-celled filaments. Tetrasporangia develop in sori in subcentral medullary areas and are often distinguishable from carpospores under a microscope, as tetrasporangia are cruciately cleaved into tetraspores (Hommersand *et al.*, 1993). Gigartinaceae tetraspores are either released through a pore in the wall or by extrusion of the entire sorus (Hommersand *et al.*, 1993). Cruciate cleavage is a characteristic of the Gigartinaceae (Hommersand *et al.*, 1993). Cystocarps are immersed in the thallus hardly protruding from the surface and do not have an ostiole (Anderson *et al.*, 1997).

Mazzaella capensis is one of the two species of *Mazzaella* that are endemic to the cool Benguela marine province (Bolton and Joska, 1993). Although *Mazzaella capensis* occurs on the rocky shore at Kommetjie, it is psamm~~a~~ophilic, typically occurring on sand-inundated rocky shores. Its Pacific relatives live on rocky shores (Bolton and Joska, 1993). The closest of these relatives is the Chilean *Mazzaella laminarioides* (Bory) Fredericq (Hommersand *et al.*, 1994). Using molecular sequencing techniques

Hommersand *et al.* (1994) showed that *Mazzaella capensis* is identical to *Mazzaella laminarioides* in the *rbc*L tree within the limits of the sequence resolution. It is likely that *Mazzaella laminarioides* originated in South America and was possibly distributed to the western Cape, South Africa in comparatively recent times (Hommersand *et al.* 1994). *Mazzaella laminarioides* forms erect fronds from a crustose basal disc (Gómez *et al.*, 1991). The formation of fronds from a perennating basal disc improves the survival of the population and maintains its competitive dominance in the rocky intertidal shore (Gómez *et al.*, 1991). Although frond formation in *Mazzaella laminarioides* is continuous, there is a seasonal pattern (Gómez *et al.*, 1991). Bolton and Joska (1993) showed that there was also a seasonal change in biomass and density in *Mazzaella capensis* from a minimum biomass of *ca* 1150g.m^{-2} and a density of *ca* 50 plants m^{-2} in early winter (June), with a massive increase to *ca* 1150g.m^{-2} and *ca* 500 plants m^{-2}in October. The Canadian *Mazzaella cornucopiae* (Postels *et* Ruprecht) Hommersand is a more distant relative (Hommersand *et al.* 1994) and has a mat of fronds that are very densely packed (Scrosati and De Wreede, 1998).

In Chile large amounts of *Mazzaella laminarioides* are harvested in response to the high demand for carrageenan (Santelices and Norambuena, 1987). Due to this interest in its carrageenan-producing potential, a lot of research has been done on the Chilean and Canadian species of *Mazzaella* (see, Santelices and Norambuena 1987; Ang *et al.* 1990; Gómez, *et al.*1991). The South African species, *Mazzaella capensis*, however is poorly researched and its potential as a useful carrageenophyte has not yet been determined. Although the populations of *Mazzaella capensis* are too scattered to be economically harvested (Bolton and Levitt, 1992), the yield of carrageenan varies from 30- 42% (Bolton and Levitt, 1992) with a maximum in early spring (Bolton and Joska, 1993). These values are slightly lower than the carrageenan yields of the commercially exploited *Mazzaella laminarioides* in Chile, which overall has an average of 41% with a minimum of 35% and a maximum of 50% (Santelices and Norambuena, 1987). The similarity of *Mazzaella capensis* carrageenan yield to that of *Mazzaella laminarioides* suggests that mari- or tank-culture might be possible as this has proved promising with other *Mazzaella* species (*e.g. Mazzaella cordata*) in North America (Waaland 1976, 1981).

Apart from the commercial carrageenophyte potential of *Mazzaella*, it has been used in the study of patterns and mechanisms of gametophyte / sporophyte alternation (Bolton and Joska, 1993). In the Gigartinales kappa(κ)-carrageenan is produced by the gametophyte and lambda(λ)-carrageenan by the tetrasporophyte (Garbary and De Wreede, 1990) The difference between κ-carrageenan and λ-carrageenan is the position and number of the sulfate group on the molecule (Craigie and Leigh, 1978). The reaction of κ-carrageenan with resorcinol-acetyl produces a red colour and λ-carrageenan produces no colour or a slight pink tinge (Garbary and De Wreede, 1990). This test can be used to identify tetrasporophytes and gametophytes, as they are isomorphic and can not be differentiated visually. The life-history phase, however, can be determined by examining at the microscopic structure of the gametangia and the immature spores.

The ratio between gametophytes and tetrasporophytes (G:T ratio) is an important descriptor of population structure within the Gigartinaceae and it may vary with species, season, or site conditions (Craigie and Pringle 1978; Dyck et al. 1985; Lazo et al. 1989; Luxoro and Santelices 1989; De Wreede and Green 1990; Dyck and De Wreede 1995). Patterns in biomass and isomorphic phase appear to be regulated by geographic factors such as the position on the shore and seasonal changes, although the presence of a basal disc permits spatial and temporal maintenance (Gómez *et al.*, 1991). Pruning frequency appears to be a limiting factor that influences the regeneration of the basal disc of *Mazzaella laminarioides* (Gómez, *et al.*, 1991). Natural regeneration of the *Mazzaella laminarioides* fronds on the surface of the crust is not a uniform process, but rather one of patchy formation (Gómez, *et al.*, 1991).

There is a lot of disagreement over the terms, crust and holdfast, and their functions. The terms used in this project will be defined specifically. A crust is the tissue that forms after the germination of a spore. This tissue is photosynthetic, it attaches the sporeling to the substrate, it takes up nutrients from the surrounding water and there probably is translocation of substances between the various cells. Once the upright (frond) has been initiated the tissue technically serves as a holdfast as it receives photo-assimilates and nutrients from the fronds and during this stage its crust function remains dormant. As the tissue can survive without the presence of fronds and is able to survive sand inundated conditions, it will be defined as a crust-holdfast complex. A plant will be defined as the crust-holdfast complex together with the thallus tissue of the fronds.

Growth and development of form are fundamental phenomena of life as is metabolism with which they are intimately connected. There is continuous modification of form in the development from a single cell to a multicellular organism. Organisms pass through a juvenile stage to maturity, and in the formation of reproductive cells the organism becomes the initiator of new cycles of development. The fundamental phenomenon of development is the growth of the single cell. Growth is understood to be an irreversible modification of form, bound up with physiological activity of the protoplasm, usually involving an irreversible increase in volume or mass of the cell (Harder *et al.* 1965). For the development of a multicellular organism from a fertilised egg (carpogonium in the case of Rhodophyta) continuous cell division must accompany the growth process. The direction and intensity of cell division can have a profound effect on the form generated. Most of the growth in the Rhodophyta is by an apical cell or apical cells resulting in uniaxial or multiaxial development (Bold and Wynne 1978). Growth in the Gigartinaceae is monopodial, multiaxial and is composed initially of converging adaxial and radiating abaxial pseudodichotomously branched filaments (Hommersand *et al.* 1993). There is an apical meristem that extends along the margin, or is dispersed over the thallus surface (Hommersand *et al.* 1993).

Growth development and productivity of seaweeds are controlled by certain internal factors and environmental factors such as irradiance, temperature, nutrient availability, and water movement (Lobban, *et al.* 1985). Within a certain range increase in temperature can cause in increase in the rate of growth. There is an optimal window for growth with a minimum temperature below which there is no growth, an optimal temperature range at which the growth rate is at a maximum, and above which growth decreases and eventually ceases.

Growth needs an energy source and autotrophic organisms produce this energy via photosynthesis. Light is of fundamental importance because of its role as an energy source in photosynthesis. Light also influences growth by its photoperiod, quality (wavelength) and intensity. The response of photosynthetic rate to light intensity is plotted in P *versus* I (irradiance) curves, where photosynthetic rate increases linearly with irradiance until it approaches a plateau at saturating irradiances. At low light intensities photosynthetic rate is limited by the light reactions of photosynthesis (photochemical reactions) (Lüning, 1990). The slope of this curve is largely independent of temperature and cell metabolism. At saturating irradiances, the maximum photosynthetic rate (P_{max}), is a function of the dark reactions of photosynthesis. These dark reactions are enzymatic and therefore temperature as well as other factors which influence cell metabolism will influence P_{max}. The irradiance at which the initial slope and P_{max} intersect is a convenient reference point on the curve, and is usually taken as the irradiance of the half light saturation constant (Darley, 1982). The response of growth rate to irradiance is similar to the P *versus* I curve (Darley, 1982), except growth is usually saturated at lower irradiance levels (Lüning, 1990).

The possible causes for the inability of algal growth rates to keep up with high photosynthetic rates may be due to sub-optimal nutrient levels in the water and inhibition of non-photosynthetic, light-dependent enzyme reactions at irradiance levels below those inhibiting photosynthesis (Lüning, 1990). It would be an obvious adaptive strategy for algae to modify their photosynthetic response to ambient light intensity so as to maximize their growth potential under various light conditions. This response, called sun-shade or light-shade adaptation, may be accomplished by modifying the light reaction (changing the initial slope) and/or the dark reactions (changing P_{max}) (Darley, 1982).. Typical shade adaptation in culture conditions involves ~~in~~ an increase in the chlorophyll content per cell with a resultant steeper initial slope and lower I_k. Within a species there may be ecotypic differentiation in light saturation of growth (Darley 1982, Lobban *et al.* 1985). Sporelings are often released in the shade of the adults and should therefore be able to grow at lower irradiances. Adults growing in different levels on the shore could also be differentiated in respect of their light saturation of growth.

Many seaweed species have been shown to be clonal (Ang and De Wreede 1992; Martínez and Santelices 1992; Flores-Moya *et al.* 1996; Scrosati and De Wreede 1998). Offshoots of clonal plants are termed ramets (Lawrence, 1995) and the intermingling of different ramets makes genets difficult to identify. Therefore the dynamics of ramets of vascular plants have been more commonly investigated (de Kroon 1993). For monospecific stands of even-aged, non-clonal plants that are actively growing, density dependent mortality of the smallest plants occurs due to the increased crowding; this is known as self-thinning (Weller 1987; Weiner 1988; Begon *et al.* 1996). Ramets of non-clonal vascular plants there fore undergo self-thinning during growth. The lack of self-thinning in some clonal plants is related to physical integration among the ramets (Hutchings 1979; de Kroon and Kalliola 1995). Some populations of clonal algae behave like non-clonal terrestrial plants in the sense that the individual genets also undergo self-thinning once crowded conditions are reached (Black 1974; Dean *et al* 1989; Ang and De Wreede 1992; Flores-Moya *et al.* 1996).

Chilean *Mazzaella laminarioides* displays self-thinning among fronds (Martínez and Santelices 1992) but some clonal seaweeds such as *Mazzaella cornucopiae* do not undergo self-thinning even at the highest recorded natural densities (Scrosati and De Wreede, 1998).

The main objectives of this project were to investigate some aspects of the biology of this potentially useful carrageenophyte. The development of *Mazzaella capensis* from spores in culture was documented. The structure of the crust and the crust-holdfast complex was investigated and this included determining the life history-phase of fronds and the distribution of small and large fronds on each crust-holdfast complex. The ratio of tetrasporophytes to gametophytes in the field was determined for early winter (May) and Spring (October), to see if there is any change in the pattern similar to the increase from 20% to 80 % gametophytes that was found by Bolton and Joska (1993) at a sand inundated site. The sporeling growth at different irradiances was investigated and compared to sporeling growth data from other species in the Gigartinaceae: *Gigartina polycarpa* (Kützing) Setchell *et* Gardner and *Sarcothalia stiriata* (Turner) Leister.

METHODS

Habit of *Mazzaella capensis* on shore and its grazers

The study site, the rocky shore at Kommetjie (34°08.1'S, 18°18.6E, approximately 30km from Cape Town) was visited initially in May (late autumn) and then on a monthly basis until October (Spring). Although empirical data was collected only in the months of May, August, and October. The general growth pattern, physical and ecological factors that could influence the occurrence, and distribution of *Mazzaella capensis* were observed monthly, to determine if there was any pattern or aspects that might have proven interesting and/or relevant to the rest of the project. The grazers that were observed actively grazing on *Mazzaella capensis* and changes in abundance over the study period (May- October 1998) were noted.

Growth and development of spores in culture

In the laboratory, the development of the crust from spores was documented photographically. Four fertile thalli were collected from the rocky shore at Kommetjie. Each thallus was assayed using the resorcinol-acetal test (Garbary and De Wreede, 1990) to determine the life-history phase.

A disc was cut from each frond and blotted with paper towel and placed in a test tube, to which 2ml of resorcinol-acetal reagent was added. The test tubes were incubated in a water bath at 90°C for 60 seconds. When they were removed the colour of the reagent mixture was noted. No colour indicated that the blade was tetrasporophytic and pink to dark red colour indicated that the blade was gametophytic. The resorcinol stock solution was 9ml of, 150mg of resorcinol diluted in 100ml of distilled water, added to 100ml-concentrated HCl. The acetal stock solution was 1ml of 0.1ml of acetal in 10ml distilled water diluted in 25ml of distilled water. The resorcinol-acetal reagent was prepared by diluting 1ml of acetal stock solution in 100ml of resorcinol stock solution. Resorcinol-acetyl reagent produces a red colour in the presence of κ-carrageenan, which is produced by gametophyte. The lack of colour or a slight pink tinge indicates that κ-carrageenan is absent and that the thallus is a tetrasporophyte (Garbary and De Wreede 1990).

The entire surfaces of the thalli were scraped with a scalpel and then washed to remove epiphytes. Each frond was washed in filtered seawater, placed in Povidone Iodine distilled water solution ($5g.l^{-1}$) for two minutes, then washed in distilled water for three minutes and rinsed again in filtered seawater. Each frond was cut into small pieces (*ca.* 5mm by 3mm) of which eight were placed on coverslips in each of four sterile crystallizing dishes and one was placed in each of the 36 compartments in each of the four sterile clear plastic trays. The plastic trays were *ca* 1cm x 1cm and the whole tray was covered by a clear plastic lid. The use of both crystallizing dishes and plastic trays was to determine the best method for culture.

The solution in the growth vessels contained one-third strength enriched seawater medium (Provasoli, 1968). To prevent diatom growth, 2ml of saturated aqueous solution of germanium dioxide (GeO_2) was added to 1litre of the enriched seawater medium which was then sterilized in an autoclave.

The growth vessels were kept in a room with a constant temperature of 15°C under a light bank of four cool white fluorescent tubes which provided irradiance of $60\mu\text{mol.m}^{-2}.\text{s}^{-1}$ and checked daily. After spore release, which took 24 – 48 hours, the thalli were removed to prevent contamination. The spores were photographed using a camera on a Leica inverted compound microscope and the period of their development documented until uprights had developed substantially (28 days after spore release).

Life-history phase composition of crusts and ratio of life history phases on shore

Crusts with fronds were randomly collected from Kommetjie in late autumn (May) and were analyzed to determine whether the fronds on each crust-holdfast complex were entirely gametophytic, or tetrasporophytic or had both phases present on one crust-holdfast complex. The positions of the remaining crust-holdfast complexes was noted within the study area and compared to the shore in spring (October) to see if any persisted. The phase of four fronds from each of the 19 crust-holdfast complexes sampled was tested by using resorcinol-acetyl reagent, using the same procedure described previously. The fronds of a gametophyte and a tetrasporophyte were examined to determine whether there was any visible difference between the phases. Cut sections were examined under a microscope to test the validity of the resorcinol-acetyl test. Tetrasporangia develop in sori in subcentral medullary areas and have distinct cruciate cleavages. Cystocarps are immersed in the thallus hardly protruding from the surface and do not have an ostiole and single spores are distinguishable. Thirty-five plants were randomly collected in spring (October) and the life history phase of one frond on each crust-holdfast complex was determined using the resorcinol-acetal reagent in the same manner as previously described.

Frond dynamics

Forty-three (19 in August and 24 in October) whole plants were removed from the rocks at Kommetjie. A scalpel was used to ensure that the whole crust-holdfast complex was taken. The outlines of the crust-holdfast complex were traced onto graph paper and the area (mm^2) was determined by counting the blocks representing each crust-holdfast complex. The structures of the crust-holdfast complexes in terms of the distribution and the total number of large and small fronds on each crust-holdfast complex were noted,. Fronds less than two cm in length were defined as small fronds and those larger than 2cm as large fronds. Most of the small fronds were between half and one cm long and the large fronds were generally more than 6cm long but can be up to 30cm long. Total number of fronds per crust was regressed against the area of the crust and a correlation co-efficient was determined for the data. These occurrences were noted and the life history phase tested (the single fronds being included). The mean density (±SE) of large and small fronds on the crust were compared using a *t*-test for independent samples. The occasional occurrence of single large fronds with a very small crust-holdfast complex was found in October. These fronds were not included in the analysis as it was considered likely to be a single plant formed from a crust produced by one spore.

Irradiance and growth

Two fertile thalli (one gametophyte and one tetrasporophyte) were collected from the rocky shore at Sea Point (34° 55'S, 18° 23'E). The thalli were treated and washed in the same manner as the thalli collected from Kommetjie. The thalli were cut into small pieces (about 5mm x 3mm) and one gametophyte and one tetrasporophyte were placed on either side of a slide (two replicates per dish) in sterile crystallizing dishes. The solution in the crystallizing dishes contained sterilized one-third-strength enriched seawater medium (Provasoli, 1968). To prevent diatom growth 2ml, of saturated aqueous solution of germanium dioxide (GeO_2) was added to 1litre of the enriched seawater medium. The solution was changed weekly.

The crystallizing dishes were placed on racks in a large water bath maintained at 18°C. The sporeling growth was measured at six different irradiances (10, 30, 60, 80, 120 and $200\mu mol.m^{-2}.s^{-1}$) with two crystallizing dishes per light level. These irradiances were achieved by placing a frame which was subdivided into six sections with varying layers of shade cloth. Each light level was measured with a light meter, at the height of the crystallizing dish before the tank was filled. A bank of incandescent white lights (Osram 400W) provided light.

After 24 hours, the sori of the gametophyte had released spores and the thalli were removed. The tetraspores were immature and did not release any mature spores. These thalli were removed and replaced by gametophytic thalli that released more spores within three hours. The slides were examined every 24 hours and sporeling growth was recorded by measuring the sporeling crust diameter of ten single sporelings per replicate at each irradiance level. A calibrated eyepiece micrometer installed in a Leica inverted microscope was used to measure each sporeling. Measurements were stopped after the sporelings initiated uprights, since further observations of crust diameter would no longer represent total sporeling growth.

The mean increment per day (referred to as the relative growth rate or RGR) for each replicate was calculated as in Anderson *et al.* (1996) by the normal compound interest formula:

$$RGR = (\sqrt[n]{D_2 / D_1} - 1)100$$

where:

n = number of days
D_1 = initial diameter
D_2 = final diameter.

The RGR data for each irradiance level were fitted to the hyperbolic tangent function $P = P_{max} \tanh(I/I_k)$ of Chalker (1980,1981). Mean (±SE) was plotted on to the fitted curve. ANOVA was used to determine statistical differences among irradiance levels.

The mean time taken by 30 spores to develop uprights in the first set of culture experiments at 15°C and 24 hour light-day of $60\mu mol.m^{-2}.s^{-1}$, was compared to mean time taken by 30 spores to develop uprights in the light intensity experiment that was at 18°C and irradiance level of $60\mu mol.m^{-2}.s^{-1}$. A *t*-test for independent samples was performed on the data to determine whether the time taken in each treatment was significantly different.

All Statistical analyses were performed using Statistica (Statsoft 1996). The assumption of equality of variances among groups was verified using Levene's test. The assumption of normality was assessed visually using a normal probability plot (Zar, 1984). Post hoc comparisons were computed using Tukey HSD.

RESULTS

Habit of *Mazzaella capensis* on shore and its grazers

The study site at Kommetjie is a rocky shore that has no incidence of sand inundation. The top of the shore has loose rocks on a bed of sandstone, towards the mid-shore there are large flat rocks with many crevices and rock pools. The lower shore and sublittoral zone are dominated by dense populations of seaweeds growing on the essentially flat rocky substrate that has crevices and raised rock outcrops. This site has a rich seaweed flora. *Mazzaella capensis* occurs higher up the shore compared to other littoral seaweeds such as *Gigartina* spp. and *Aeodes orbitosa* (Suhr) and is found more frequently on the higher lying rocky outcrops. *Mazzaella capensis* has however also been seen to occur in the low-lying areas between the *Aeodes orbitosa* and *Gigartina* spp. The crust holdfast complex of *Mazzaella capensis* is an area of slightly raised dark brown tissue that becomes thicker when the fronds develop. The crust-holdfast complex bears erect juvenile fronds which are initially cylindrical with a dome-shaped apex which grow into small strap-like flattened fronds. There is no distinct division between the crust-holdfast complex and each of its fronds.

The densely packed mat of fronds of *Mazzaella cornucopiae* is very different to *Mazzaella capensis* which has similar growth habits to *Mazzaella laminarioides* with fronds forming from a crustose basal disc (crust-holdfast complex). The crust-holdfast complex of *Mazzaella capensis* varied in size from 11-1400mm^2. There is a strong correlation between the crust area (mm^2) and the total number of fronds on a crust (Fig. 1, $r^2=0.70$, n=12, $p<0.05$).

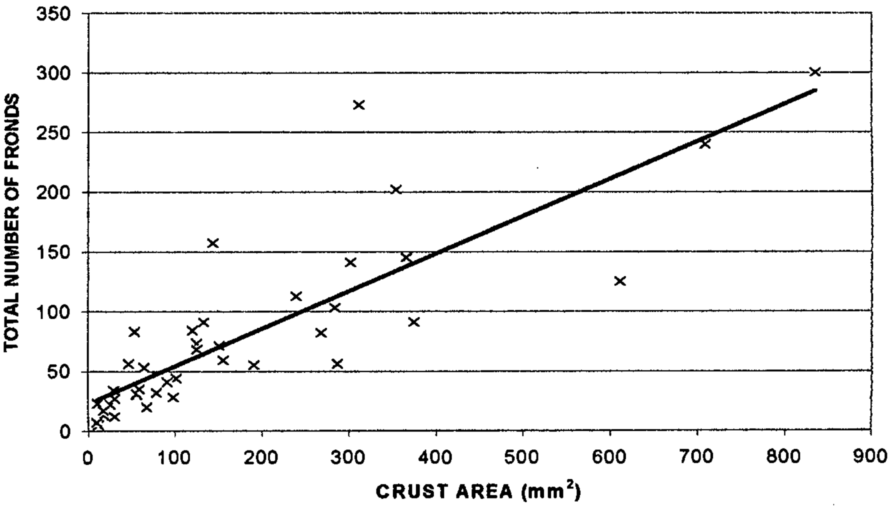


Figure 1: Total number of fronds on *Mazzaella capensis* crust-holdfast complex *versus* crust-holdfast complex area.

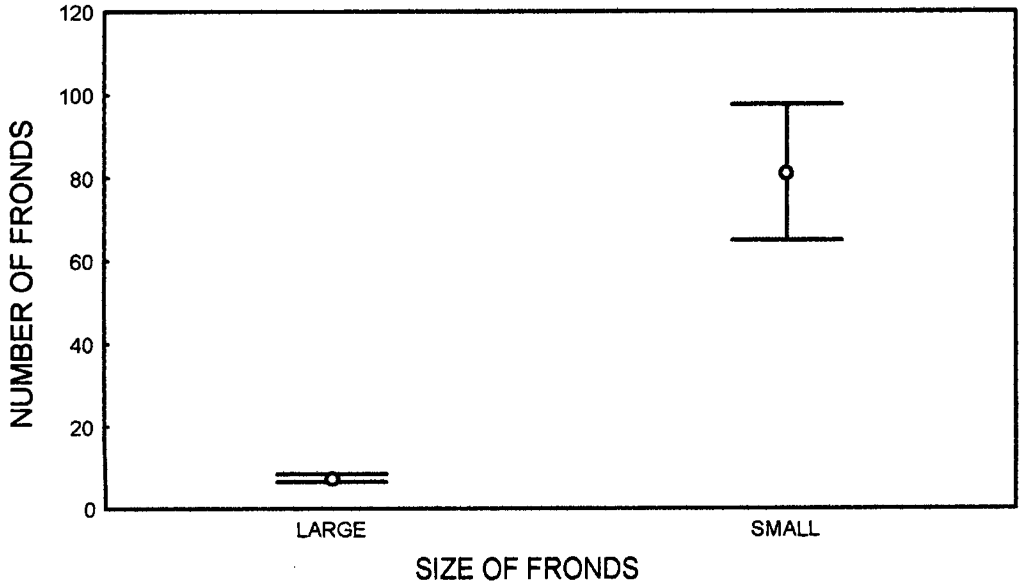


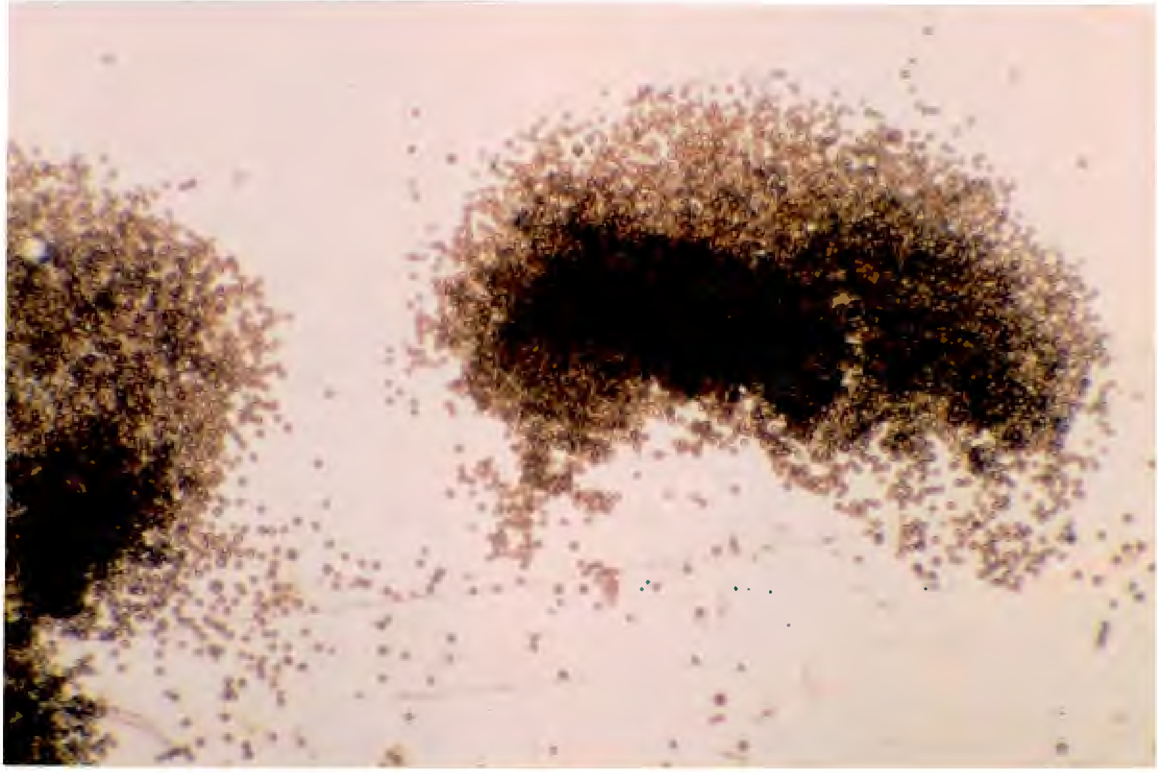
Figure 2: Mean (±SE) number of large and small fronds on *Mazzaella capensis* crust-holdfast complex.

There was no notable difference in the average number of fronds found on each crust-holdfast complex taken at August and October although the average size of the small fronds was larger in October (*ca* 1 - 1.5cm) than in August (*ca* 0.5 – 1cm). The average size of the large fronds was smaller in October (*ca* 6 - 10cm) than in August (*ca* 10 - 20cm). There average ratio of large to small fronds in August and October was not significantly different (t-test for independent samples, $t=0.428$, df=38, $p>0.05$) therefore they were grouped together. There is a significant difference between the number of large and small fronds per mm^2 of *Mazzaella capensis* crust (Fig. 2, t-test for independent samples, $t=-4.438$, df=81, $p<0.0001$). The average ratio of large to small fronds was 7:49. The large fronds are concentrated in the middle of the crust-holdfast complex and numerous small fronds were visible round the edges and a few small fronds were found in the gaps between the large fronds

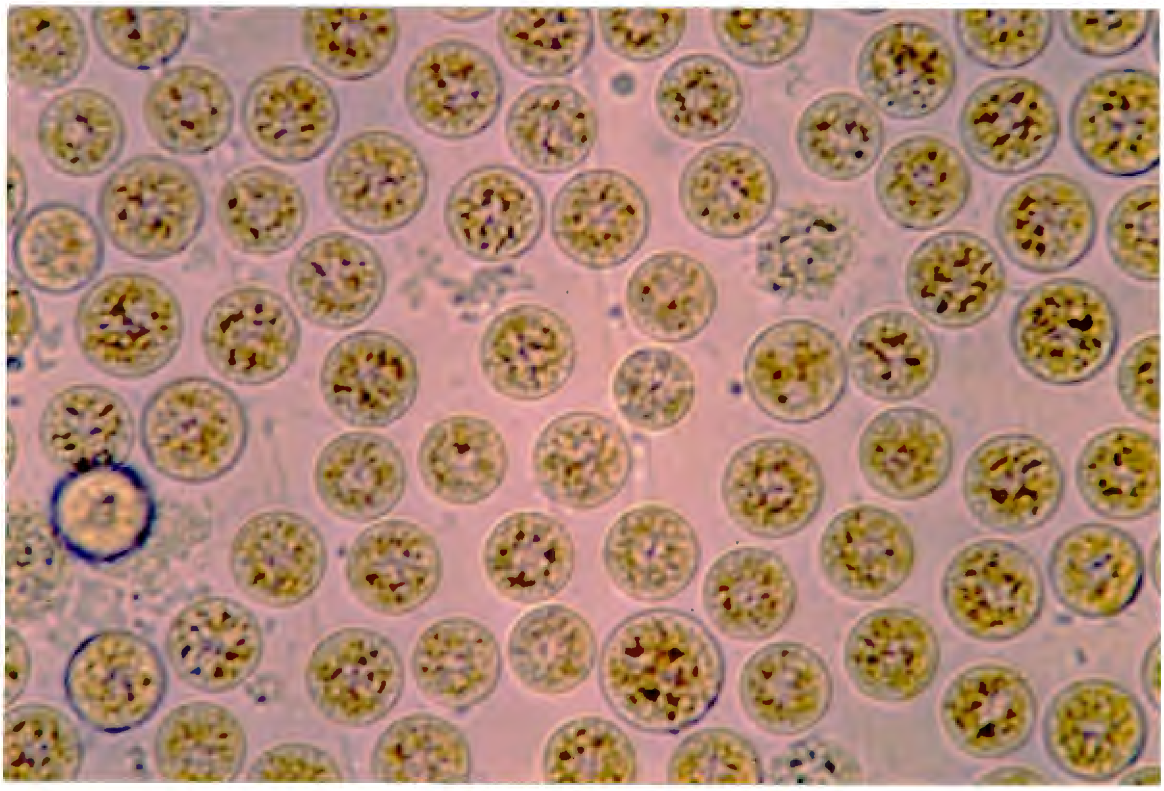
The isopods and amphipods found grazing on *Mazzaella capensis* were *Exosphaeroma varicolor* Barnard, the variegated spherical isopod and *Hyale grandicornis* (Kröyer), the seaweed amphipod. Various species of limpets, including *Patella granatina* Linn., *Patella granularis* Linn. and *Helcion pruinosus* (Krauss) were found grazing both the fronds and the crust-holdfast complex.

Spore growth in culture

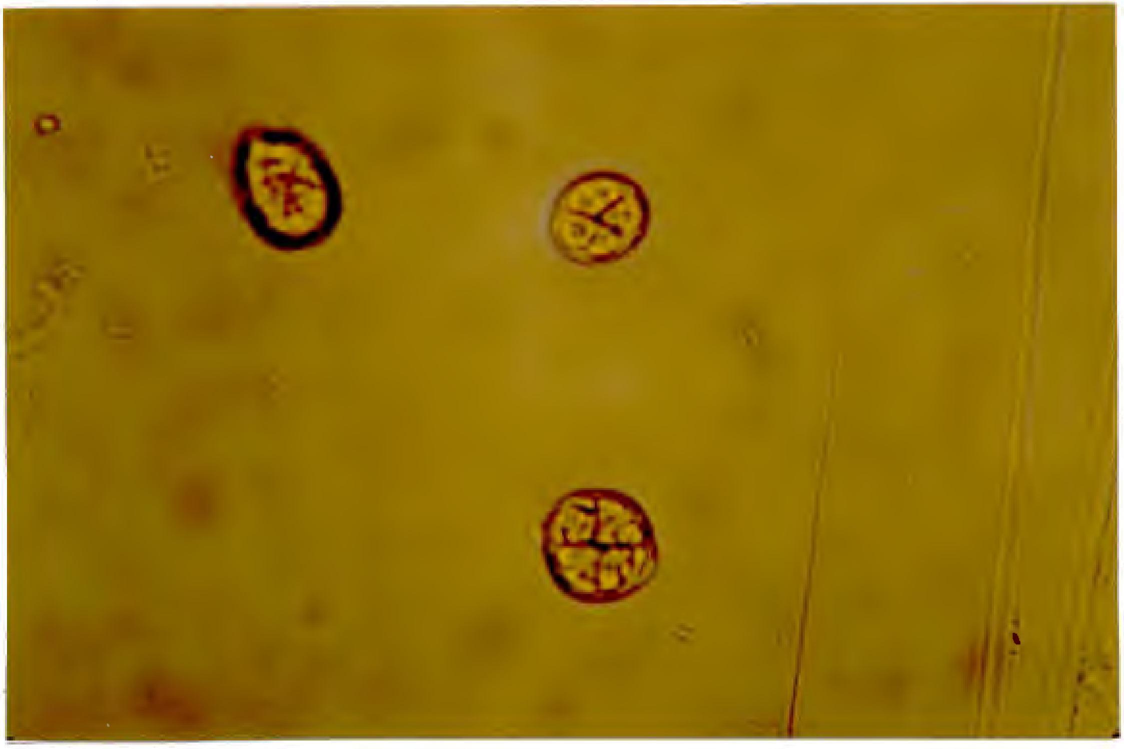
The life-history phase of the thalli for the four replicates in each culture method was determined by a resorcinol test. There were two sets of gametophyte thalli replicates and two sets of tetrasporophyte thalli replicates in the growth vessels for each culture method. The tetrasporophytes released most of their spores within 48 hours whereas the gametophytes released only a few spores and the thalli started to decompose and had to be removed after 4 days. The crystallizing dishes proved to be the best method of culture as the growth medium was easy to change and glass was less susceptible to scratches and bacterial growth. Spores were released in two different ways, either the whole sorus was extruded from the thallus or the sorus was not extruded and a regularly spaced "pavement" pattern of spores was observed on spore release (Fig. 3 (b)). The developmental sequence of the attached tetraspores (juvenile gametophytes) (Fig.3 (c), and (d)) shows cellular division resulting in the formation of circular crusts. Figure 3(e) shows the development of rhizoid-like projections that occurs if the spores do not attach properly. The crust discs coalesced if they were close to one another (Fig 3 (f), (g) and (h)). Both the coalesced circular crusts and individual attached crusts eventually start producing uprights 20-28 days after germination (Fig. 3(i) and(k) respectively). There was no formation of thick typical crust-holdfast complex in laboratory conditions after five weeks.



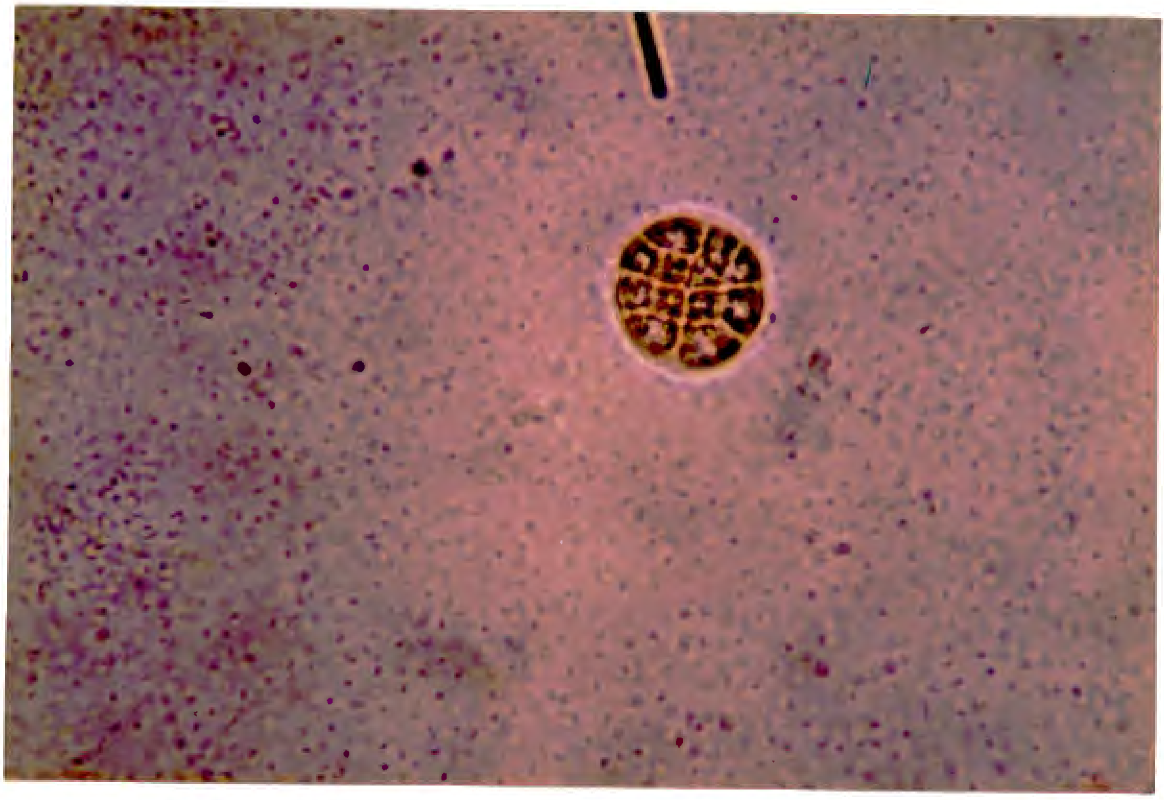
(a)



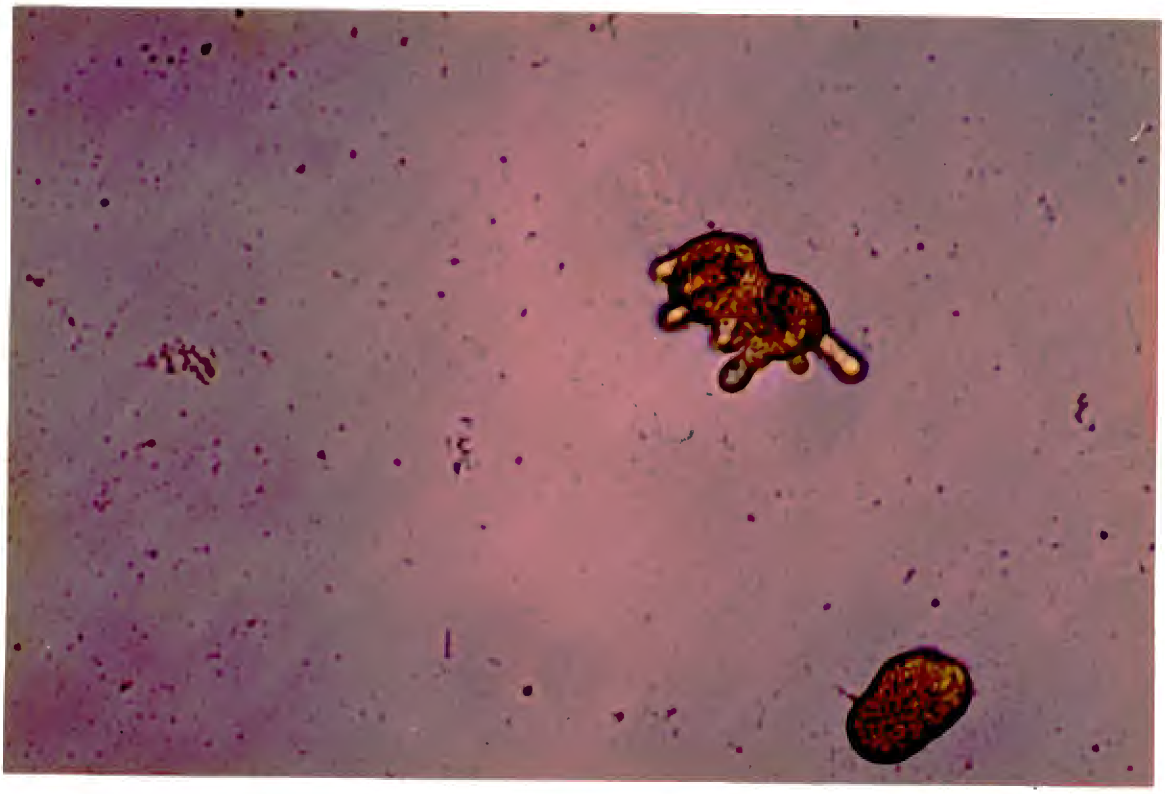
(b) 11µm



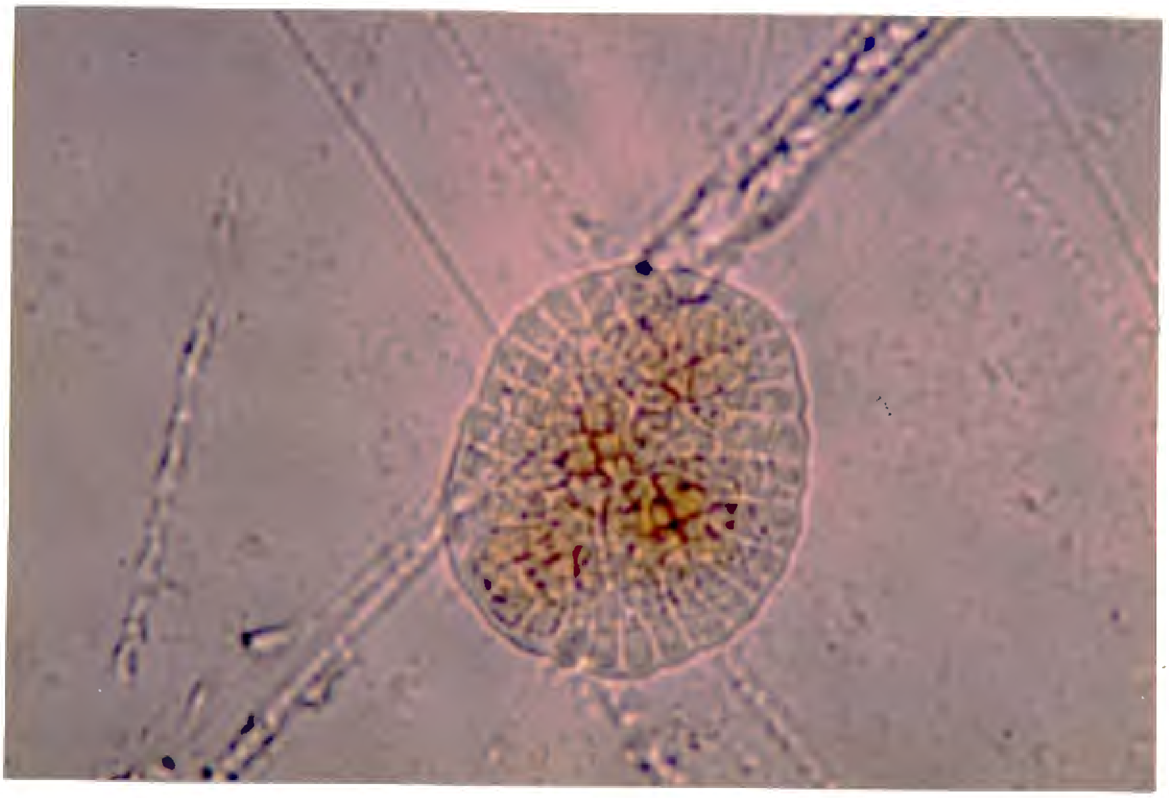
(c) 111µm



(d) 11μm



(e) 22.5μm



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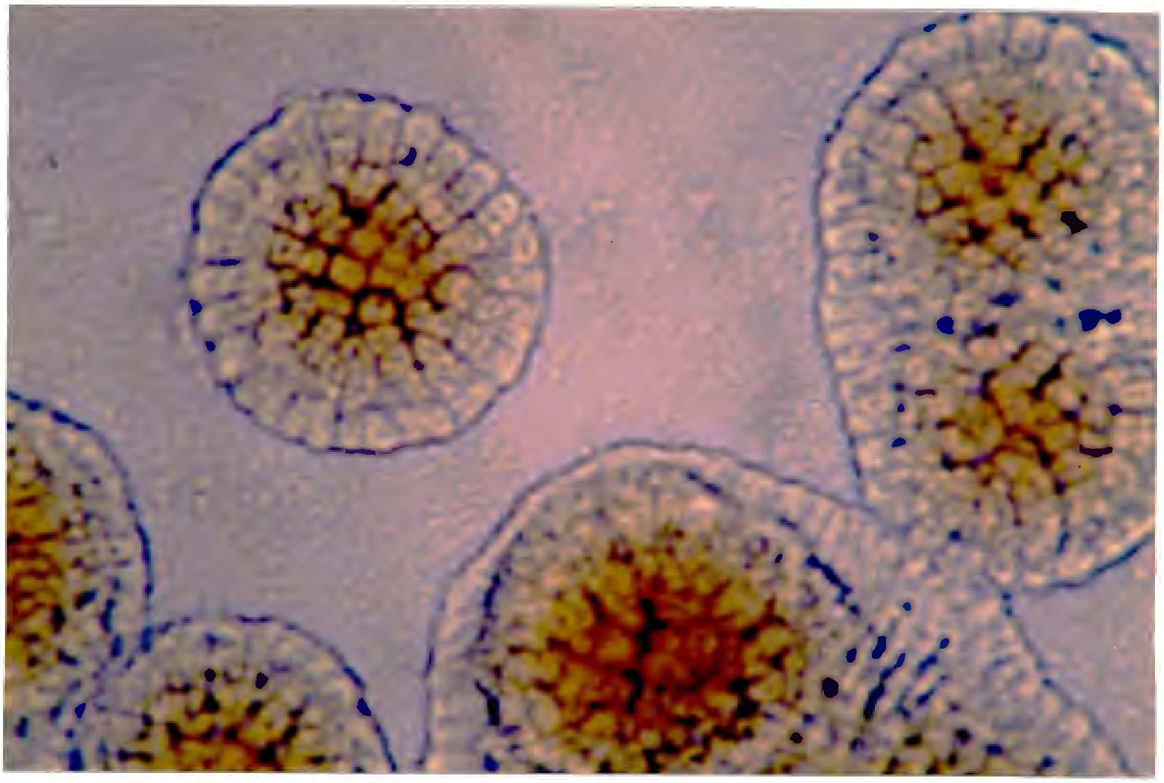

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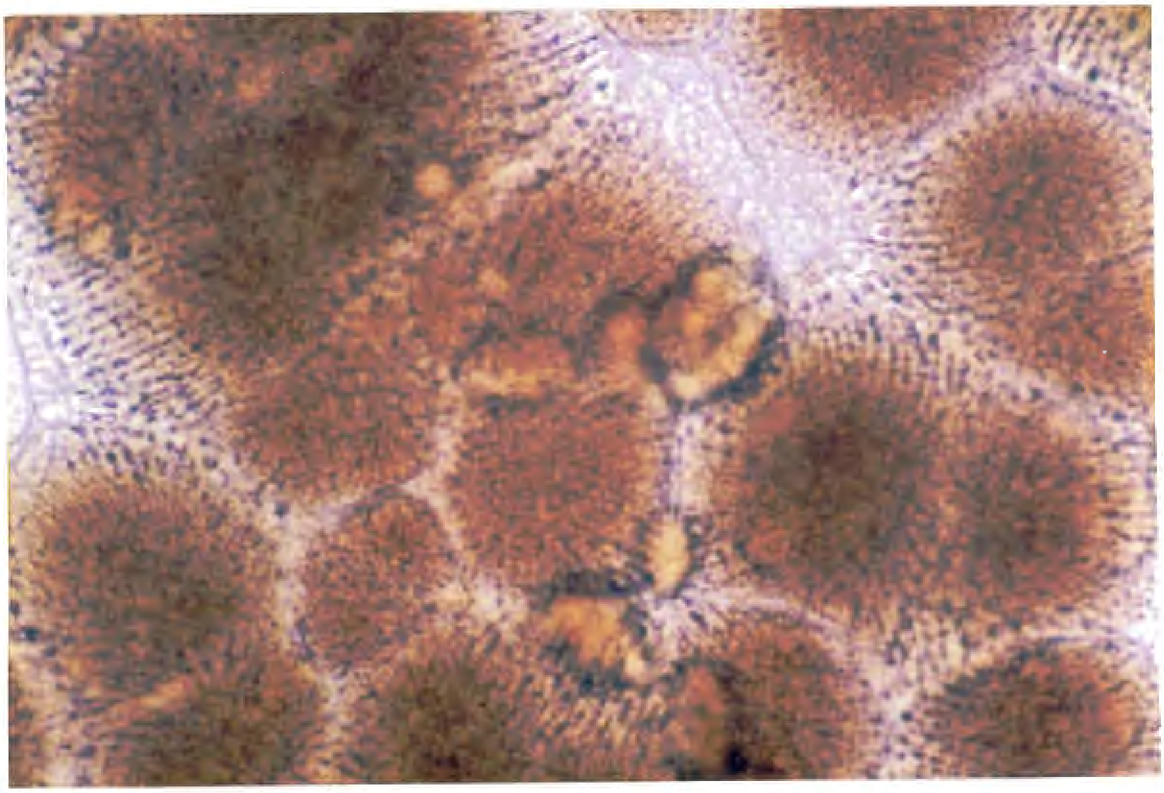

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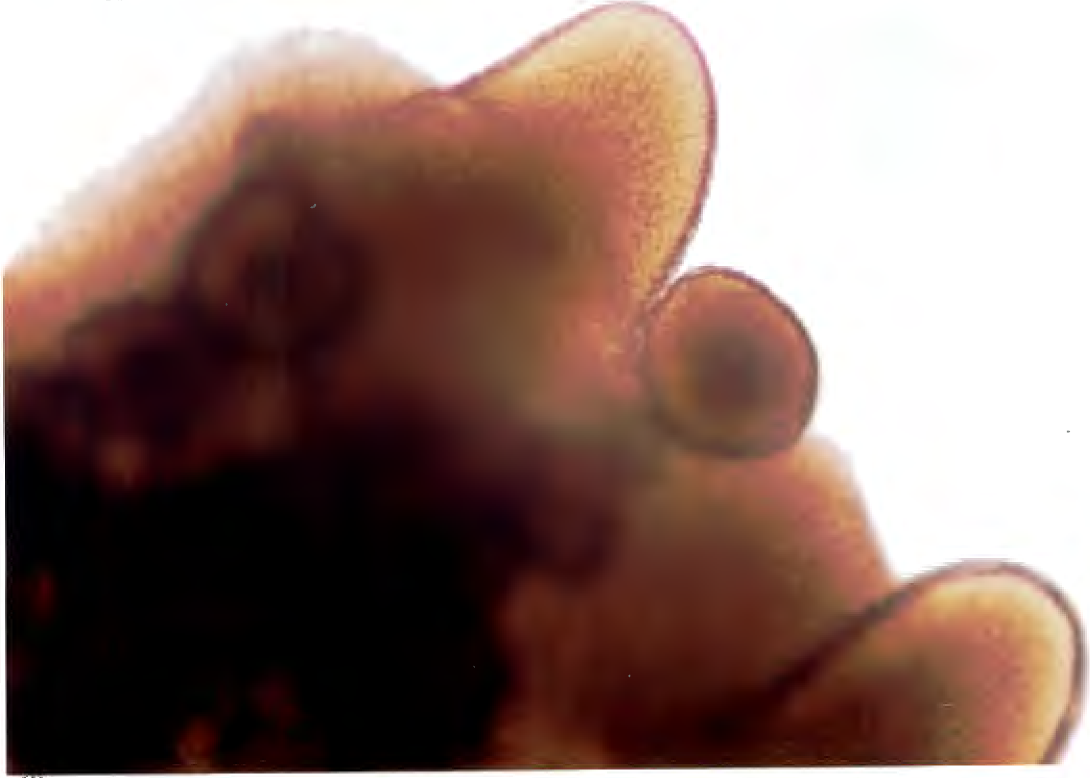
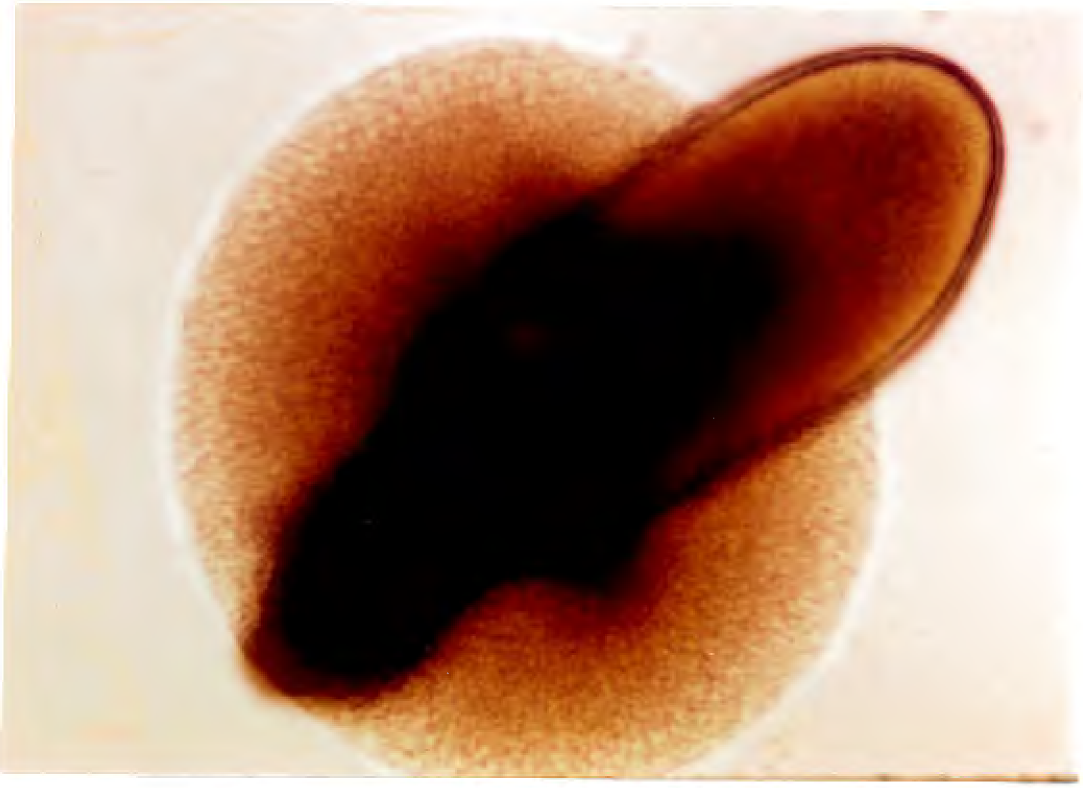


(i) 22.5μm

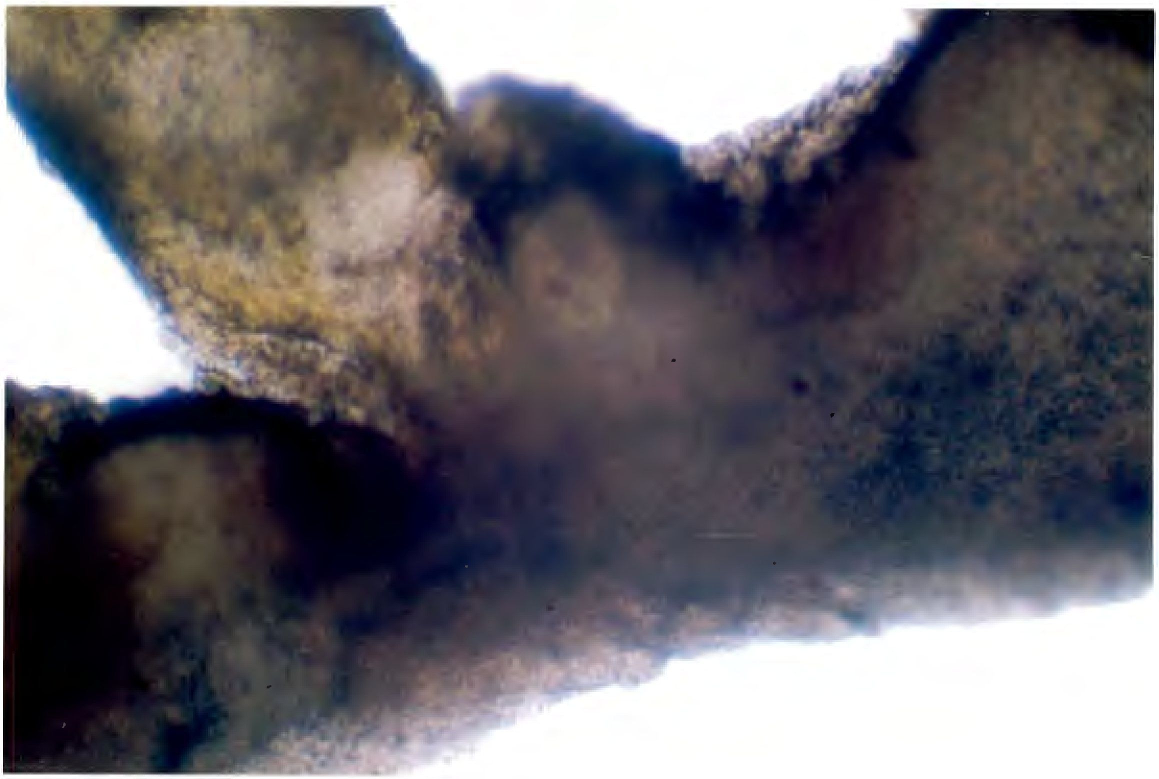


(j) 22.5µm

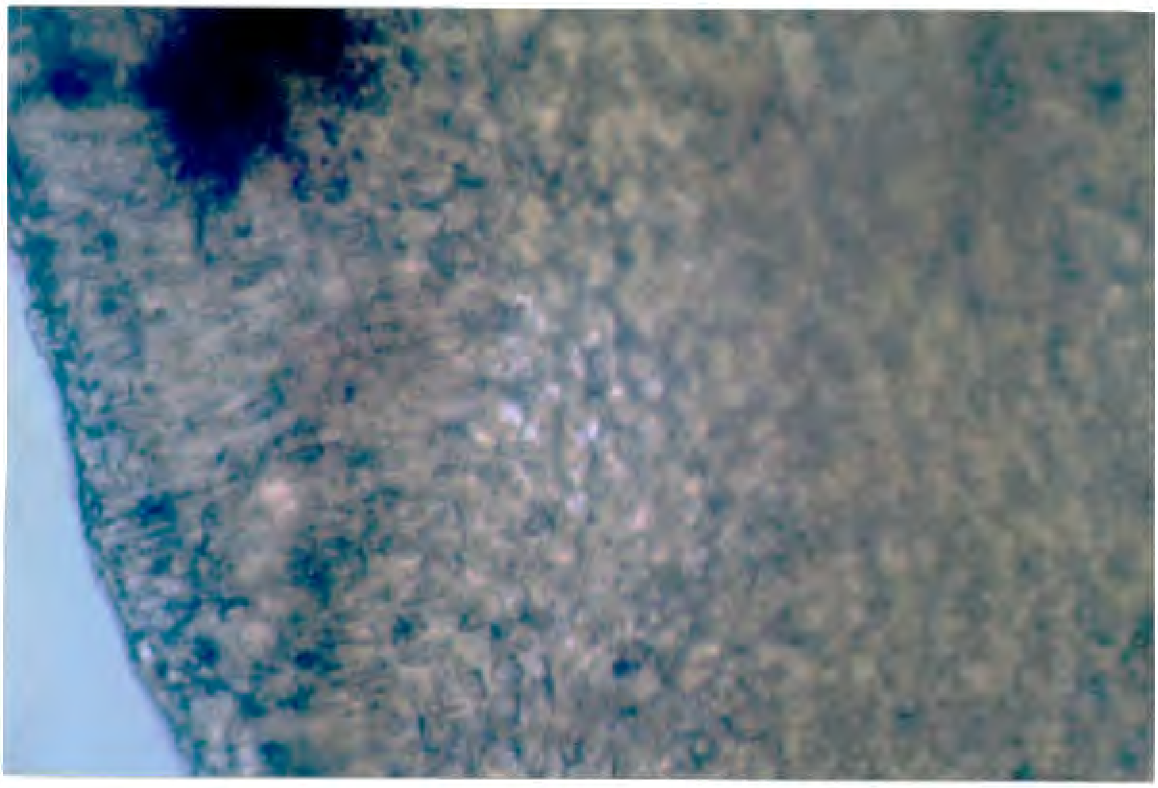
Figure 3: Developmental sequence of sporelings from release to initiation of uprights. (a) Extrusion of the entire sorus (x4) (day1). (b) Spores released in "pavement" pattern (x40) (day 1). (c) Germinated spore: 4-cell stage (x40) (day 2). (d) Germinated spore: 12-cell stage (x40) (day 3). (e) Unattached spore developing rhiziod-like projections (x20) (day3) (f) Developed crust: multi-cellular (x40) (day 8). (g) Coalescing crusts (x40) (day 8). (h) Coalescing crusts before uprights develop (x20) (day 14). (i) Coalescing crusts with developing uprights (x20) (day 20). (j) Developed crust with developing upright (x20) (day 23).

Structure of the crust-holdfast complex from the field

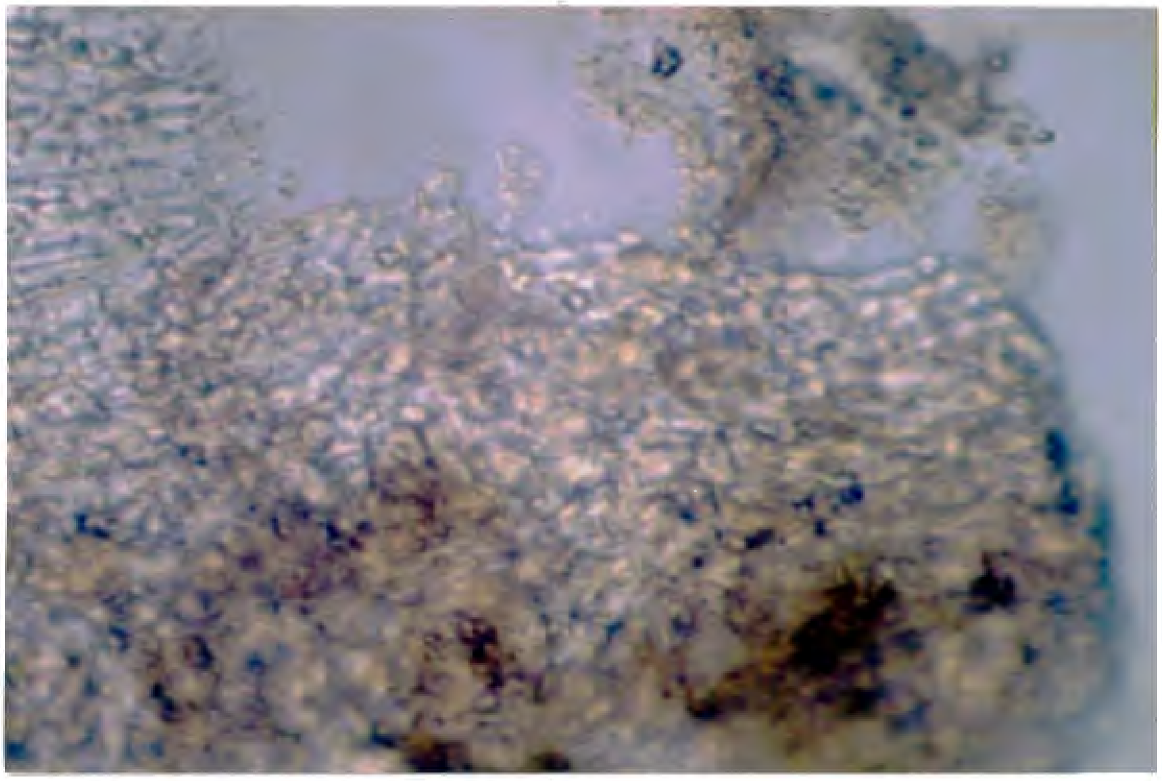
The crust-holdfast complex that occurs in the field (Fig. 4 (a)) is different from the laboratory-cultured crust of five weeks, as it is distinctive to the naked eye, is thick and consists of many layers of cells. This may be due to growth conditions in culture, or more likely that the crust-holdfast complex formation is secondary and takes longer to form. The tissue of the crustose holdfast complex (Fig. 4) consists of an indistinct medulla and a cortex of radiating filaments of small cells (Fig. 4(b)). The medulla (Fig. 4(c)) is an extension of the radiating filaments of the cortex (Fig. 4(d)) where the cells become distorted and more elongated with many more spaces between the filaments and the individual cells. The developing uprights are an extension of the crust-holdfast tissue (Fig. 4(e)). The area occupied by the crust-holdfast complexes varies from 11-1600 mm^2, whereas the thickness is between one and two mm, irrespective of size.



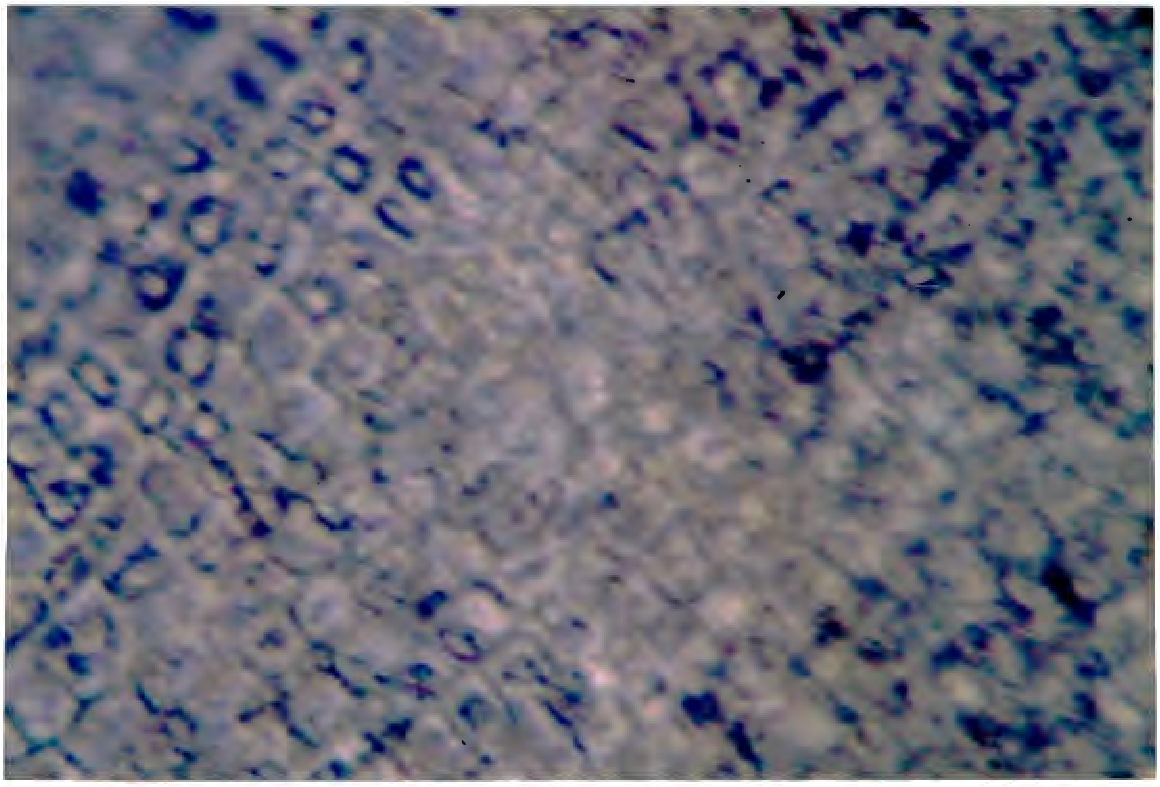
(a) 307μm



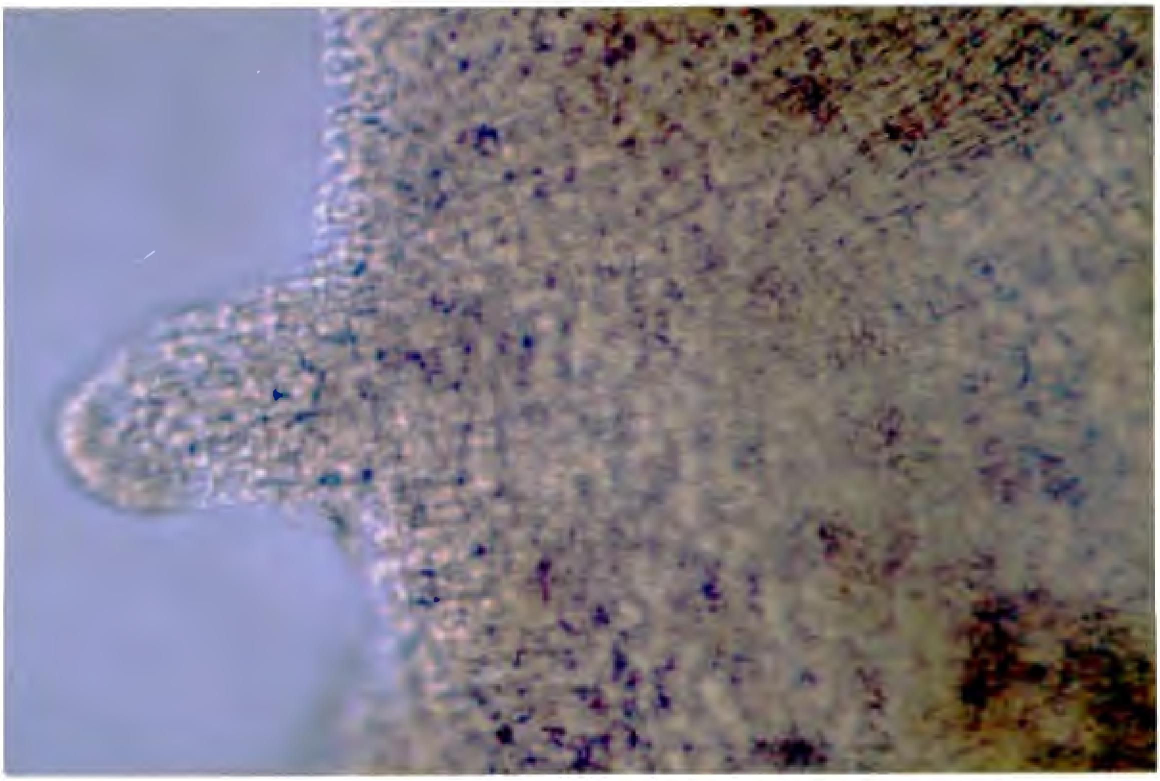
(b)



(c)



(d) 11μm



(e) 22.5μm

Figure 4: (a) Section of crust-holdfast complex (x4), (b) Section of section of holdfast tissue showing the medulla and cortex regions. Detail of tissue of (c) outside cortex (x40) and (d) inside medulla (x40). (e) Close up of upright formation (x20).

Life-history phase composition of crusts and ratio of life history phases on the shore.

All crusts sampled had monophasic fronds. The shore at Kommetjie was dominated by tetrasporophytes with a gametophyte:tetrasporophyte (G:T) ratio of 1:17 in late autumn (May 1998) and by gametophytes with a ratio of 32:3 in spring (October 1998). Some of the tetrasporophytes that were present in winter persisted into the summer months but the increase in gametophyte recruits resulted in a reversal of dominance. The individual large fronds with a very small crust-holdfast complexes found in October were tetrasporophytes.

Spore disc growth in relation to irradiance.

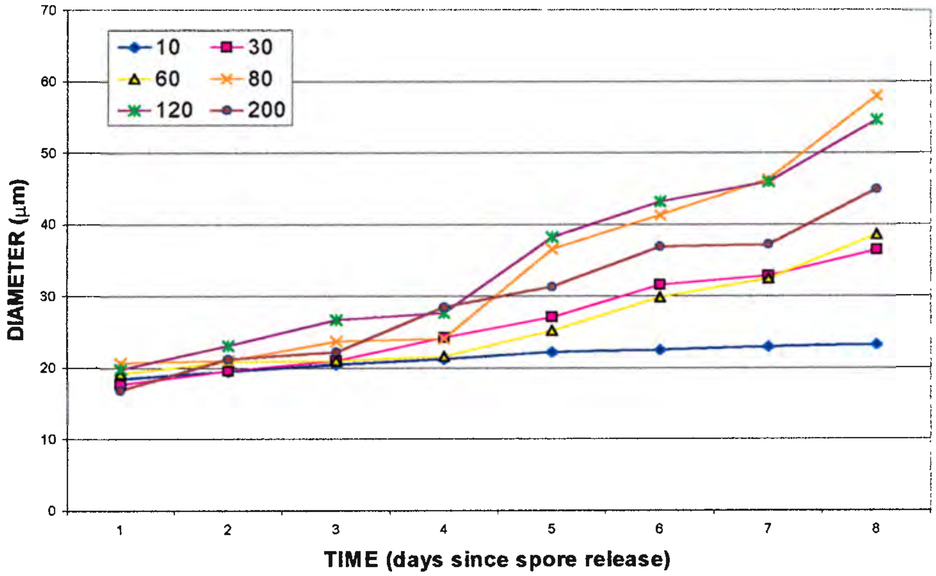


Figure 5: *Mazzaella capensis* sporeling diameter increase since spore release at different irradiance levels (μmol.m^{-2}.s^{-1}) shown in legend.

Note that the size of spores are not equal at day 1. The best growth is at an irradiance level of 80μmol.m^{-2}.s^{-1}. Uprights developed after 7 days in some of the spores at 60, 80, 120 and 200μmol.m^{-2}.s^{-1}, and after 8 days at 30μmol.m^{-2}.s^{-1}. At 10μmol.m^{-2}.s^{-1}, growth of the sporelings was minimal and no uprights developed during the period of observation.

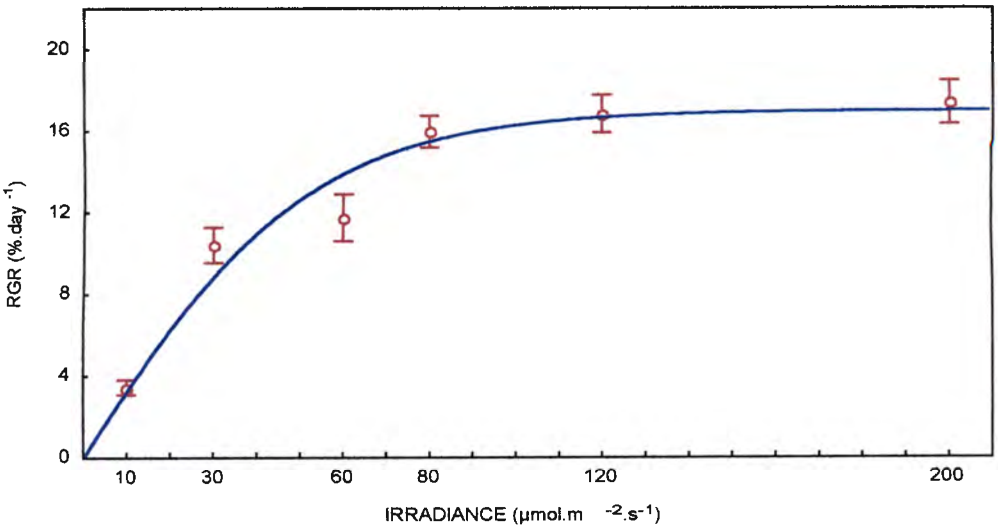


Figure 6: Relative growth rate between day 1 and day 8 *versus*. irradiance (*P-I*) curve for *Mazzaella capensis* sporelings. Mean(±SE) RGR at each light level used.

There were significant differences among growth rates at various irradiances (Fig.6, one-way ANOVA, F=34.788, df=5, 17, p<0.05). Growth at 10μmol.m^{-2}.s^{-1} was significantly slower than growth at all other irradiances (Tukey HSD test, p<0.05). Growth at 30μmol.m^{-2}.s^{-1} was significantly faster than 10μmol.m^{-2}.s^{-1} (p<0.05) and significantly slower than growth at 80, 120 and 200μmol.m^{-2}.s^{-1}. There was no significant difference between growth at 30μmol.m^{-2}.s^{-1} and 60μmol.m^{-2}.s^{-1} (p>0.05). Growth at 80, 120 and 200μmol.m^{-2}.s^{-1} were not significantly different (p>0.05) The I_k value is the half saturation constant (Darley, 1982) for *Mazzaella capensis* was 52μmol.m^{-2}.s^{-1} and therefore the growth saturation point was 104μmol.m^{-2}.s^{-1}.

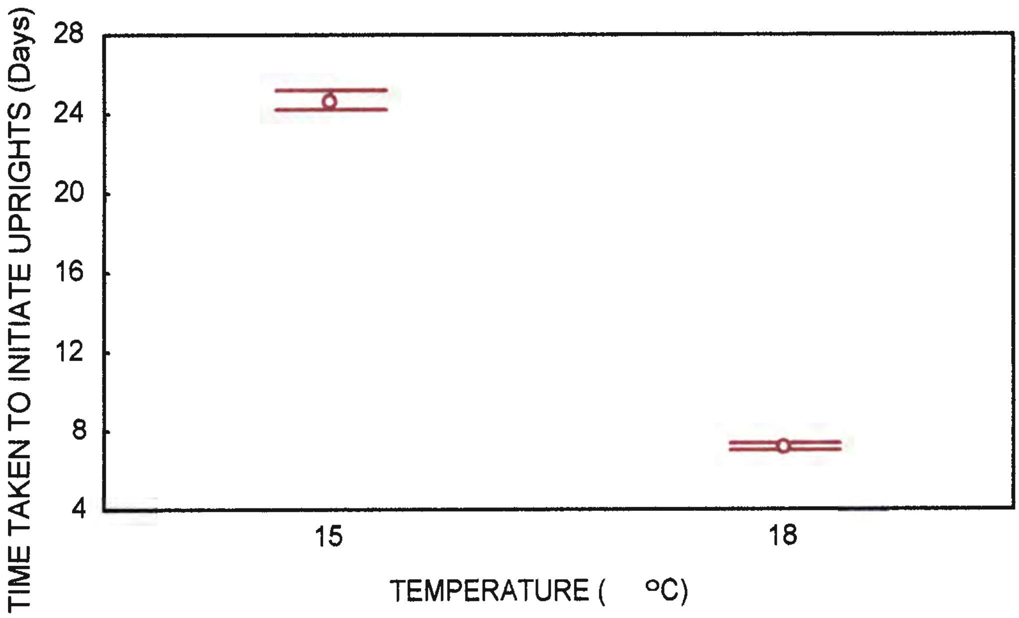


Figure 7: Mean(±SE) number of days taken by *Mazzaella capensis* spores to develop uprights at 15°C and 18°C at an irradiance level of 60μmol.m^{-2}.s^{-1}.

The number of days taken for each of the 30 spores to develop uprights in the first set of development experiments at 15°C and 24hour light day of 60μmol.m^{-2}.s^{-1} was significantly slower than that at 18°C at 60μmol.m^{-2}.s^{-1} (Fig. 7; *t*-test for independent samples, t=34.350, df=58, p<0.05).

DISCUSSION

Development and life history phases

The habit of *Mazzaella capensis* on the rocky shore at Kommetjie is quite different to the extensive, continuous, very thin crust-holdfast complex which covers the rocks at the seasonally sand inundated shore of Wireless Road *ca* one km away, where Bolton and Joska (1993) conducted their study. However, at Kommetjie *Mazzaella capensis* is similar to *Mazzaella laminarioides* (Gómez, *et al.*, 1998) in that it has separate crusts-holdfast complexes that are interspersed among the rocks and other seaweeds. This contrasts with the Canadian *Mazzaella cornucopiae*, which forms dense mats of fronds (Scrosati and De Wreede, 1998). The fronds on each *Mazzaella capensis* crust-holdfast complex are all the same life history phase. From observations of spore development in the laboratory it was noted that spores are released by the sorus onto the surface below. Tetraspores are either released through a pore in the wall or by extrusion of the entire sorus (Hommersand *et al.*, 1993). Sometimes the spores are released in the distinctive pavement pattern, which ensures that each spore has an area of substrate on which to attach. This pattern is probably a result of the spores being released through the pore and spread out as they sink slowly to the substrate where they are evenly distributed due to their spherical shape (which doesn't allow them to settle on top of each other). There might be some sort of repulsive force between the individual spores induced by electrostatic interactions, or it might be a result of the gelatinous extrusions in which the spores are released that keeps them separate from each other. When the whole sorus is dropped by the parent and there is no agitation, the spores remain in that group. The spores on the bottom are able to attach to the substrate. The spores on top still germinate and often coalesce into the bottom layer or otherwise they develop unevenly with rhizoid-like projections and float around in the medium.

The development of *Mazzaella capensis* sporelings at 15°C and an irradiance of $60\mu mol.m^{-2}.s^{-1}$ for 24hours a day, was significantly slower than that at 18°C with irradiance levels above $30\mu mol.m^{-2}.s^{-1}$. This suggests warmer temperatures promote faster growth rates in *Mazzaella capensis* sporelings, but this is to be expected even of cold adapted plants as an increase in temperature promotes an increased rate of reactions. As *Mazzaella capensis* is endemic to the west coast, a lower temperature optima for growth should be expected as the sea surface temperatures throughout the Benguela system are around 13 -15°C (Shannon, 1985). Bolton and Anderson (1997) concluded that the temperature conditions of the west coast (minimum monthly mean 11.5°C; maximum monthly mean 14°C; and annual mean 12-13°C) were somewhat intermediate between the criteria used to define cold and warm temperate. Corresponding to this "cool" seawater temperature, the temperature optima of some of the west coast seaweeds have shown growth optima at temperature between 15°C and 20°C. For example, *Ecklonia maxima* (Osbeck) (Bolton and Levitt, 1985) and *Laminaria pallida* Greville ex J Agardh (Branch, 1974). As *Mazzaella capensis* is an intertidal seaweed the sporelings may be exposed to heating from solar radiation at low tide and therefore might have a higher temperature optimum than the adults as they are much smaller and have less area for cooling. In a study of Caribbean seaweeds, Pakker *et al.* (1995) found that the temperature tolerances of species investigated from three separate biogeographic groups were related to the temperature extant during their origin and dispersal history.

The G:T ratio of *Mazzaella capensis* on the Kommetjie shore alternated with the season. Tetrasporophytes being dominant in early winter, and newly recruited gametophytes were dominant in the spring and early summer. The newly recruited plants were not visually distinguishable from the old plants. This pattern seems similar to that of *Mazzaella capensis* at the seasonally sand-inundated rocky shore of Wireless road (Bolton and Joska, 1993). Some of the tetrasporophytes that were present in winter were still there in the summer months but the increase in gametophyte recruits caused the phase ratio to switch. The rapid switch from a tetrasporophyte dominated shore to a gametophyte dominated shore suggests that most of the macroscopic plants and perennating crust-holdfast complexes were tetrasporophytes. A possible seasonal trigger could have induced the production of haploid spores and the development of gametophytes in spring. Another explanation for this switch could be that perennating tetrasporophytes were continually producing haploid spores but environmental conditions or grazing pressure did not allow for the spores to mature, germinate and develop.

Grazing of the large fronds by amphipods, isopods and limpets and even the grazing of the crusts by limpets was especially heavy in winter. This observation is consistent with that observed by Griffin (1998) on the same shore. The growth of sporelings and the plant itself could be limited by the increased grazing pressure in the winter months. The effect of grazing pressure on *Mazzaella capensis* could be limiting regeneration in a similar fashion to the manner in which the pruning frequency (human harvesting) of *Mazzaella laminarioides* in Chile influence regeneration (Gómez *et al.*, 1991). A lower total biomass of seaweed was observed in late autumn (May) than in spring (October) and this may be the reason for increased grazing pressure on the seaweeds that remain. In spring the biomass increased and the grazing damage to *Mazzaella capensis* was reduced.

The crust-holdfast complex of *Mazzaella capensis* was able to survive without the initiation of uprights It has photosynthetic pigments concentrated in the upper layer and storage cells throughout the tissue (concentrated mainly on the bottom). Physical coalescence of the crusts during their development and the lack of division between the tissue of the crust-holdfast complex suggests that there may be clonal organization within the plant. Benefits of a clonal existence include translocation of photo-assimilates and nutrients, and frond crowding which offers protection against desiccation, irradiance, herbivory and wave action (Scrosati and De Wreede, 1998). In culture each crust produced from one spore developed one upright. These are genetically identical to the crust. Future work could be done on determining the genetic identity of the fronds on large crust-holdfast complexes to see if they are identical clones or whether they are genetically distinct, it would be expected that the tetrasporophytic plant would have more gentically distinct ramets than the gametophytic plant since tetraspores are formed by meiosis and carpospores are formed by mitosis.

Although the area of the crust is correlated to the number of fronds, the size distribution of the fronds on the crust-holdfast complex with a few large fronds in the middle and many smaller fronds around the edges, suggests that the plant undergoes self-thinning among the fronds. It seems feasible that nutrients, photo-assimilates and other growth promoters are mainly directed to the few large fronds. As the plant develops, the small fronds are able to grow larger due to the productivity of large fronds. Although the average size of the small fronds increased from August to October, there was still an uneven distribution between the number of large and small fronds on a crust-holdfast complex.

The occasional occurrence of single large fronds with a very small crust-holdfast complex is probably due to germination of a single spore isolated from other spores with which it could coalesce. A mass release of spores would result in them attaching in close proximity of each other making the probability of a large crust developing due to coalescence greater.

Light saturation values of photosynthesis and growth (Table 2) have been determined for a number of species of eulittoral and sublittoral algae. Populations of *Gigartina polycarpa* (Kützing) Setchell *et* Gardner occurring in both the littoral (Levitt, 1993) and sublittoral (Levitt and Bolton, 1990) are adapted to high levels of irradiance. However, photosynthetic responses and growth of spores and sporelings can be expected to differ from adult plants because of shading of sporelings by the adults (Levitt, 1998). The light saturation level of growth rate response of *Mazzaella capensis* sporelings ($104\mu\text{mol.m}^{-2}.\text{s}^{-1}$) can be interpreted as an adaptation to the low light levels experienced in the understorey of other algae, which permits maximal growth at low light saturation levels. Expected values light saturation level of growth rate for a mature eulittoral plant should be higher, in the range of 200-300μmol.m^{-2}.s^{-1} (Lüning, 1990) as they are usually sun-adapted plants in which light intensity is not a limiting factor in growth (Darley, 1982). Sun-adapted members of the Gigartinaceae have been recorded elsewhere, e.g. *Chondrus crispus* Stackhouse, *Mastocarpus stellatus* (Stackhouse) Guiry (Burns and Mathieson 1971) and *Gigartina polycarpa* (1972; Levitt 1993; Levitt and Bolton, 1990). Low saturation levels of *Mazzaella capensis* sporelings can be interpreted as an adaptation to the low light levels in the under-story of algal beds, with low light-saturation permitting maximal growth until the plant is large enough to outgrow shading by parent and neighbouring plants.

The I_k value of *Mazzaella capensis* (52μmol.m^{-2}.s^{-1}) was similar to that of *Gigartina polycarpa* and *Sarcothalia stiriata* (Turner) Leister sporelings (*ca* 50μmol.m^{-2}.s^{-1}) also grown from Kommetjie material (Levitt, 1998). The 104μmol.m^{-2}.s^{-1}saturation level of *Mazzaella capensis* sporelings supports the hypothesis that *Mazzaella capensis* sporelings are adapted to relatively low irradiances. Sporeling growth of *Mazzaella capensis* improves considerably at 30μmol.m^{-2}.s^{-1}. The daytime natural irradiances in the western Cape are commonly greater than 200μmol.m^{-2}.s^{-1} and in full sunlight can be over 2000μmol.m^{-2}.s^{-1} at the water surface and rarely less than 30μmol.m^{-2}.s^{-1}on a cloudy day, rendering the necessity of survival at low irradiances (<30μmol.m^{-2}.s^{-1}) for an extended period unlikely (Levitt, 1998). The adaptation to survive and grow in relatively low light intensities (<30μmol.m^{-2}.s^{-1}), facilitates rapid growth when light conditions improve (Levitt, 1988). Although 104μmol.m^{-2}.s^{-1} is a fairly high value for intertidal sporelings, it is low when compared to growth saturation levels of other high shore adult seaweed *species (Fucus vesiculosus* and *Fucus serratus*). Both sporophytes and gametophytes of *Mazzaella capensis* are long-lived individuals that persist on the shore as opposed to *Ecklonia maxima* gametophytes which are short-lived and reach reproductive maturity within a few days under ideal light conditions (Bolton and Levitt, 1985). This suggests that the adaptation to lower light intensities may be similar to the short-term strategy for surviving transient environmental fluctuations in the eulittoral environment of *Gigartina polycarpa* and *Sarcothalia stiriata* (Levitt, 1998), rather than the long term strategy of gametophytes of *Ecklonia maxima* that optimises survival under low light conditions for prolonged periods of time until improved light conditions facilitate rapid growth (Bolton and Levitt, 1985).

Table 1: Irradiance levels at which photosynthesis (PS) or growth (GR) are light saturated (units of μmol.m^{-2}.s^{-1}). Taken from Luning (1990).

SPECIES	**PS**	**GR**	**ALGAL TYPE**
EULITTORAL ZONE			
Fucus vesiculosus	600	300-350	Brown
Fucus serratus	500	150-200	Brown
Codium fragile	500	28	Green
Mastocarpus stellatus	460		Red
Gelidium spp. (sporelings)		50-75	Red
Devaleraea ramentaceum		50	Red
Porphyra spp.	400	30	Red
Surface phytoplankton	500		
Terrestrial sun plants	500		
SUBLITTORAL ZONE			
Sargassum polycystum	80-120		Brown
Sargassum cymosum (benthic)		150	Brown
Sargassum natans (pelagic)		200-300	Brown
Laminaria saccharina	150	70	Brown
Ulva fenestrata	80-150		Green
Chondrus crispus	180	50-100	Red
Grateloupia turuturu	80-150		Red
Phytoplankton (1% light depth)	200		
Terrestrial shade plants	60-200		
Ahnfeltia tobuchiensis	60-100		Red
Delesseria sanguinea	60		Red
Ptilota serrata	70		Red
Macrocystis pyrifera (young sporophytes and gametophytes)	70		Brown
Atractophora hypnoides		10	Red
Laminaria spp (early sporophytes)		15	Brown
Himanthothallus grandifolius (young sporophytes)		5	Brown

From Table 1 it is obvious that the irradiance level at which photosynthesis is saturated is generally much higher than growth. It seems that the difference between the irradiance levels at which photosynthesis and growth are light saturated varies between species.

The irradiance saturation points at which the growth of *Gigartina polycarpa* tetrasporophyte sporelings and *Sarcothalia stiriata* tetrasporophyte sporelings are light saturated (18μmol.m^{-2}.s^{-1} and 44.2μmol.m^{-2}.s^{-1} respectively, Levitt unpubl., pers. comm.), are lower than *Mazzaella capensis* (104µmol.m^{-2}.s^{-1}) This result is to be expected as the *Mazzaella capensis* from Kommetjie occurs higher up the shore than both *Gigartina polycarpa* and *Sarcothalia stiriata* and therefore receives more light because of longer periods exposed and the lack of shading by other species.

CONCLUSION

The rate of development of *M. capensis* sporelings in culture was found to be faster at 18°C than at 15°C. Growth rate of *M. capensis* sporelings was light saturated at 104μmol.m^{-2}.s^{-1}. In the field, there are additional factors and complex interactions which influence development of the crust and the plant. *Mazzaella capensis* appears to be a clonal plant that does not exhibit characteristics of self-thinning. The average ratio of large to small fronds on each crust-holdfast complex is 7: 39, which suggests that there the growth of the small fronds is inhibited. Each individual crust-holdfast complex and its related fronds are monophasic with respect to life history stage. The likelihood of tetrasporophyte fronds being genetically similar or even identical is high, whereas the gametophytic crust-holdfast complex will probably have genetically mixed fronds as the ? tetraspores that are produced from the gametophytes are formed by meiosis. Within the *Mazzaella capensis* population at Kommetjie, there is a switch of dominance from tetrasporophytes in early winter to gametophytes in early summer. Future studies in population and crust-holdfast complex genetics could prove useful in determining the extent of the clonal relationship. The possibility of culturing *Mazzaella capensis* from spores for mari- or tank-culture should be investigated.

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