AN ASSESSMENT OF POTENTIAL OF RED SEAWEED PALMARIA PALMATA FOR MARICULTURE IN THE IRISH SEA

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for the degree of Doctor in Philosophy

by

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ABSTRACT

The aim of this study was to grow ***Palmaria palmata*** as a potential food source. In the laboratory methods of obtaining spores, optimum conditions for spore germination and growth of sporelings under various photon irradiances, different photoperiods and in different growth media were investigated. Growth pattern of ***Palmaria*** plants were also studied under laboratory conditions. Three methods of growing ***Palmaria*** were investigated: growing vegetatively in onshore tanks using continuous flow and batch systems; seeding cords with spores and inserting fragments into cords and transplanting them to the sea.

The viability and percentage germination of spore fluctuated. Gentle filtration and addition of plant growth hormone, indole-3-acetic acid improved germination. Photon irradiance between 16 and 30 umolm^{-2}s^{-1} appeared to give best germination of about 40%. ***Palmaria*** sporelings seemed to grow best in Provasoli's ES media half strength in filtered seawater and 16:8 h (light:dark) photoperiod.

The growth pattern study showed that growth rate in length (R^L) at the tip was about twice that of the whole thallus. R^L gradually decreased from the tip to the base. R^L in width was faster at the middle. Plants with partially damaged tips showed a significant reduction in growth rate in length but not in area and weight. Plants with severely damaged tips showed a significant reduction in growth rate in terms of weight. There was a clear relationship between relative growth rate in term of length, area and weight. Area growth rate was about twice and weight growth rate was about 2.8 times the length growth rate.

Growing ***Palmaria*** vegetatively in onshore culture tanks was not economically viable in Port Erin, Isle of Man because of the high cost of pumping seawater, collecting ***Palmaria*** plants and labour for maintaining such systems. The inability of ***Palmaria*** plants to produce marginal proliferations at some time of year and the need to replace the plants periodically perhaps is one factor why ***Palmaria*** is not suitable for tank culture.

Growing ***Palmaria*** from spores and fragments on cords in the sea suffered from one common problem, fouling by unwanted seaweeds, particularly Laminariales. Growing ***Palmaria*** from spores had many problems such as low percentage germination,inability to form secure attachment on cords and inability to compete with fouling diatoms and Laminariales. Growing from fragments gave more encouraging results. Thalli produced marginal proliferations and developed into plants with sizes ranging from less than 2 to 129g.

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GENERAL INTRODUCTION

Palmaria palmata has a wide distribution throughout cooler waters of the North Atlantic and North Pacific (Guiry, 1975). It is also one of the commonest intertidal red algae in the British Isles and is found growing in a wide range of habitats. Plants reach the full expression of their potential in the shallow subtidal. Plants have been recorded from a depth of 20m but typically grow in a depth of 0-12m (Guiry, 1976).

Palmaria has been used as food for man and farm animals for centuries in coastal areas of Europe and more recently in northeastern North America (Schachat and Glicksman, 1959; Chapman, 1970; Ffrench, 1974; Brothwell, 1976). It is known to local inhabitants as "Dulse" (Great Britain), "Dillisk" (Ireland), "Söl" (Iceland), "Sol" (Norway), "Goemon à vache" (France), or "Darusu" (Japan) (Butters, 1899; Schachat and Glicksman, 1959; Levring ***et al.***, 1969; Dixon, 1973).

Although it has been used for centuries, there was a period when the utilization of dulse declined following economic affluence around the world (Davis, 1980). When grains were readily available, seaweeds were no longer in demand, the American Indians of the Pacific Coast who once gathered dulse rarely do so (Tseng, 1947).

Fortunately this trend is gradually changing. Recent public awareness about health and the emphasis on "natural food" which have been accelerating since the early nineteen-sixties has resulted in an increased market for dulse (Sirota and Uthe, 1979) and in 1977 78 tonnes were harvested off eastern coast of Canada (Fisheries Canada, 1977).

The chemical and nutritional values of ***P. palmata*** (dulse) have been reviewed by Morgan ***et al.***, (1980a). The chemical constituents of ***Palmaria*** vary according to seasonal and nutritional conditions where the plants are obtained. Fresh ***Palmaria***

contains 73-89% moisture and on a dry weight basis, 12-37% ash, 8-35% crude protein, 38-74% carbohydrate and 0.2-3.8% lipid. ***P. palmata*** also has potassium, chlorine and sodium as its major constituents and in comparison to terrestrial fruits and vegetables is a good source of iron, magnesium, calcium and iodine. It has vitamin A (as carotene) and in fresh plants vitamin C is also present in appreciable amounts. In view of this, ***Palmaria*** is potentially a high protein food source with minerals and vitamins and its protein quality rates well with vegetables of good nutritional value.

Increased demand for seaweeds in general has led to the development of extensive mariculture systems for seaweeds in the ocean (Tseng, 1981a; Holt, 1984; Dawes, 1987), in ponds (Chiang, 1981), in intensive land-based systems using tanks (Edelstein ***et al.***,1976; Braud and Delépine, 1981; Bidwell ***et al.***, 1985) or using spray culture techniques (Moeller ***et al.***, 1984; Rheault and Rhyther 1983; Indergaard ***et al.***, 1986; Lignell and Pedersén, 1986).

To cope with anticipated increase in demand for dulse, fluctuations of supply from natural grounds, due to harvesting and destruction of natural habitats has led to the need to develop mariculture of ***P. palmata.***

The aim of this study was to assess the prospects and identify various problems in cultivating ***Palmaria*** as well as to understand more about the growth pattern of the ***Palmaria*** thallus. Three different methods of cultivation were investigated. There were as follows: cultivation of vegetative plant in onshore culture tanks, and culture from spores and fragments on cords in the sea. Methods of obtaining spores, and the growth of sporelings were also investigated.

CHAPTER ONE

Spore germination and growth of *Palmaria palmata* sporelings studies under laboratory conditions

Introduction

There are two main approaches to seaweed farming: cultivation from spores and cultivation from vegetative fragments of adult thalli (Krishnamurthy, 1965). Seaweeds such as the red alga ***Porphyra*** and the brown alga ***Laminaria*** have been successfully cultivated from spores (Imada ***et al.***, 1972; Tseng, 1981a). More recently the brown seaweeds ***Laminaria*** and ***Alaria*** have been successfully cultivated in the Irish Sea from spores (Holt, 1984; Dawes, 1987).

When this method of seaweed farming was first started, suitable substrata were placed in the sea during the sporing season where natural populations occur, to collect spores. This method of collecting spores and growing seaweeds on artificial substrata is entirely uncontrollable, hence the annual production fluctuated greatly. Seeding of spores and growing seaweed on artificial substrata improved rapidly after the discoveries of life cycles by Sauvageau (1915) in species of Laminariales and Drew (1949) in ***Porphyra umbilicalis*** and when the development and requirements of spores and sporelings were better understood. Cultivation techniques were later improved with the introduction of methods for the artificial collection of spores, and seeding was carried out in a more controlled environment, resulting in an increase in seaweed production (Tseng, 1981a; Chiang,1982; Holt, 1984).

If ***Palmaria palmata*** is to be cultured by seeding spores on to cord followed by transfer to the sea, knowledge of its biology is vital. Chemin (1937) and Inoh (1939) studied the biology and development of ***Palmaria*** spores. The absence of female

Palmaria had puzzled phycologist for many years. Cytological studies (Yabu, 1971, 1976; van der Meer, 1976) demonstrated a haploid-diploid chromosome relationship between spermatangial and tetrasporangial plants. Culture studies were also carried out to find the elusive female plant and to study the biology of ***Palmaria*** plants in culture (Sparling, 1961; Prince, 1973; Guiry, 1976). There was a widespread belief that ***Palmaria*** had no sexual reproduction (Bold and Wynne, 1978). This was later discounted when the life history was discovered and proved in culture (van der Meer and Chen,1979; van der Meer and Todd, 1980) (Fig. 1.1).

Palmaria can be found in abundance in many parts of the northern hemisphere (Guiry, 1976). It has been used by man as food, fodder and fertilizer. It has been mentioned that ***Palmaria*** is also one of the very few seaweeds eaten in the western world and has been used for centuries in the coastal areas of Europe and more recently in northeastern North America. The economic potential of ***Palmaria*** cannot be denied and with aquaculture in mind research on this species is entering a new phase.

If ***Palmaria*** can be grown from spores on artificial substrata in the same way as ***Porphyra*** and ***Laminaria***, the high cost of growing it vegetatively in tanks or inserting plant fragments into cord can be avoided. There are two main prerequisites for growing ***Palmaria*** economically on cord: good germination of spores and fast growth of sporelings. Good germination means better usage of the available spores. It also means that most of the cord surfaces are covered with sporelings and offer little space for growth of unwanted species (Hanic and Pringle, 1978; Mumford, 1978). Germinated sporelings grown on cord must be big enough to withstand the rough conditions in the sea and compete successfully with unwanted species. The growth of sporelings must be fast so that the incubation period is kept to a minimum. Prolonged incubation not only increases cost but is more likely to result in weak attachment of holdfasts to the cord.

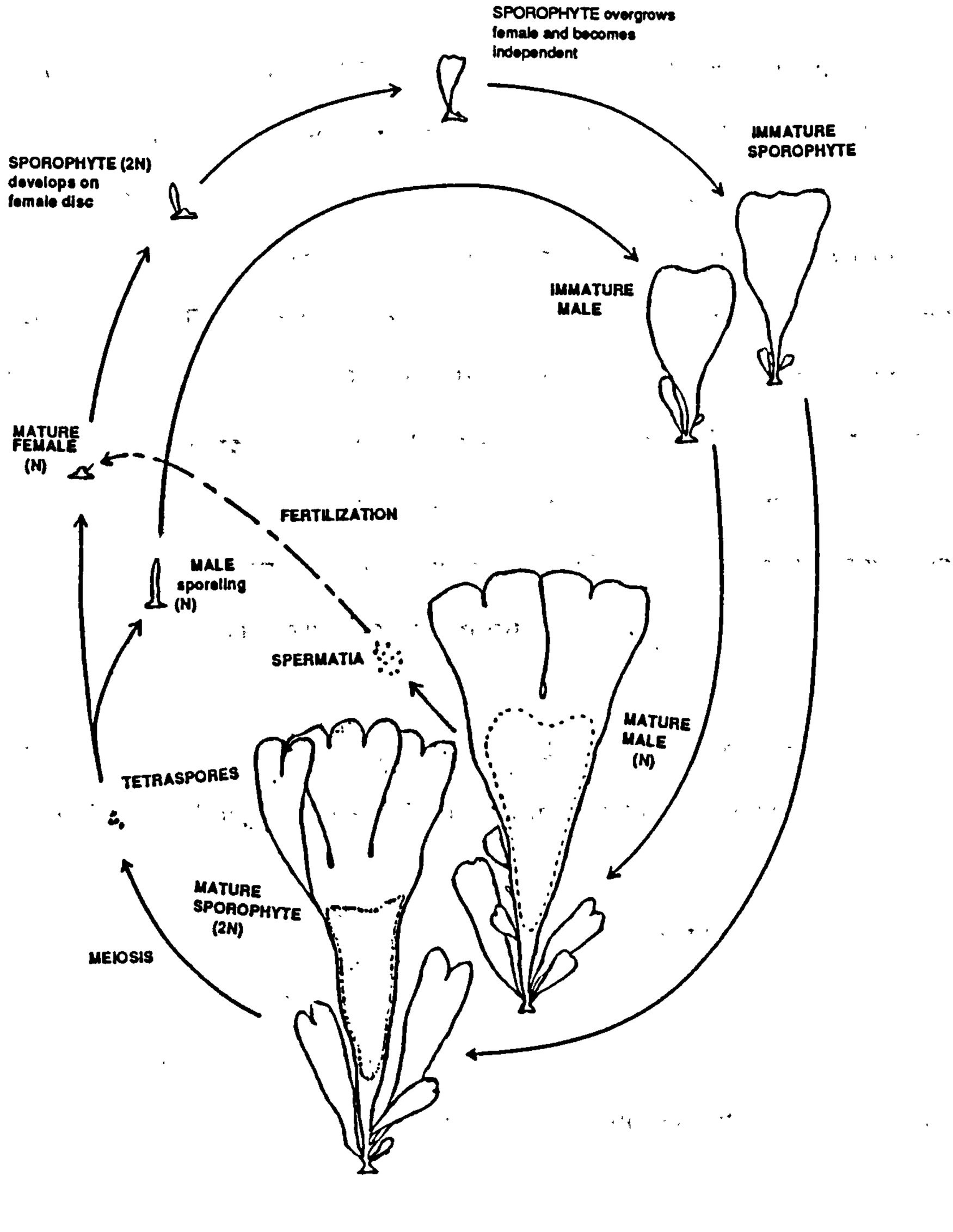


Fig. 1.1. The sexual life history of ***Palmaria palmata*** (van der Meer and Todd, 1980).

Prince (1973) found that release of spores and germination of ***Palmaria*** were successfull at 5 and 10^0C. Growth was temperature-dependent and the optimum temperature appeared to be between 10 and 15^0C. Growth of sporelings was better in complete media with trace metals and vitamins.

As the work has been carried out in different places, in natural seawater and possibly on different strains, some differences are to be expected. Germination may vary with location (van der Meer and Chen, 1979) and the quality of seawater, which affects the growth of seaweed, is known to vary considerably with location, season and depth (Johnston, 1962). The effects of hormones on seaweed in general have been reviewed by Augier (1976a,b,c, 1977a,b,c, 1978). Reports of the effects of growth hormones on germination are very scarce. Hustede (1957) reported that IAA promotes the formation and germination of zoospores in***Vaucheria.*** Since the germination was low and varied, it would be very interesting to find out whether plant growth hormones have similar effects on the germination of ***Palmaria*** spores

The general aims of this study were to find the ideal conditions for germination and growth of sporelings in Port Erin, Isle of Man. More specifically they were:

1) to find reliable methods of obtaining large quantities of spores;

2) to study the effect of growth hormones (IAA and kinetin) at various concentrations on germination;

3) to determine the effect of irradiance on germination;

4) to study the effect of different growth media in natural seawater on the growth sporelings and;

5) to study the effect of varisous irradiances and photoperiod on the growth sporelings.

Materials and methods

1.1. Algal materials

Obtaining a good supply of sporing subtidal tetrasporophyte material is difficult during the autumn and winter months when the sea conditions are often too rough for diving. To solve this problem, material was stored in tanks on land. In late October *Palmaria palmata* was obtained from the subtidal region of Port Erin breakwater.

Healthy plants free from epiphytes were selected and kept in four outdoor conical tanks, each with a capacity of 404 litre and surface area of 0.7 m^2, with flowing seawater, aerated and with a stocking density of 1.1-1.4 kg/m^2. As the level of nutrients in the sea is quite high at this time of the year, no additional fertilization was given as this might have encouraged the growth of epiphytes. From time to time spent tetrasporophytes were removed from the tanks and replaced with fresh ones.

1.2. Pretreatment of sporing tetrasporophytes

Whenever tetraspores were required, healthy looking sporing tetrasporophytes were collected from the conical tanks and rinsed with 2 micron filtered seawater several times to remove as much debris as possible.

1.3. Release of tetraspores

Release of tetraspores in the laboratory appears to require temperature shock and dim light or darkness, release occurring within 1-4 days (Prince, 1973). In Port Erin most of the release seemed to occur within 24 hours. Two ways of releasing tetraspores were investigated to determine which is more efficient.

In the first method, cleaned tetrasporophytes were laid in a white enamel tray and left in a constant temperature (CT) room at 10^0C in the dark for 2-3 hours. After that filtered seawater was poured into the tray and left overnight. The next day ***Palmaria*** thalli were removed carefully from the tray and tetraspores could be seen on the white background as deep red patches. Tetraspores were collected using a pasteur pipette. This technique was modified slightly from the one used by several other researchers (Boney ***et al.***, 1959; Sparling, 1961; West,1968; McLachlan and Edelstein, 1977; Grandy, 1984).

Initially the second technique was quite similar to the first; sporing materials was left in a dark CT room for three hours. After that about 500g of thalli were placed in a white 10 litre bucket which was filled with 2 micron filtered seawater and aerated gently overnight. The next day the aeration was stopped and the thalli were removed, gently shaking them. Left behind was a tetraspore suspension plus other suspended organic matter, which was allowed to settle for 1-$1^1/_2$ hours. Tetraspores and other heavier debris settled to the bottom while smaller and lighter debris were still suspended in the water. Most of the water was decanted and discarded leaving about $^1/_9$ of the volume.

Palmaria tetraspores are very delicate and surrounded by protective colourless mucilage with diameters ranging from 30 to 35 microns (Prince, 1973; Guiry, 1976). Extreme care had to be taken to minimize tetraspore damage. A filter consisting of PVC pipe 88mm in diameter and 60mm long, with 45 micron nylon screen attached to one end was constructed. This was submerged in a white plastic beaker to a depth of about 3cm and the remaining spore suspension was poured gently from the bucket through the filter. The 3cm height of water allowed tetraspores to pass through gently while retaining the bigger debris. The filtrate contained mostly tetraspores. The used tetrasporophytes were returned to the conical tank outside and could be used again after 10 days.

2.0. Apparatus

All culture experiments were carried out in lidded 8cm diameter flat bottom glass crystallizing dishes each containing a "seasoned" plastic petri dish 55mm diameter and 5mm deep. From preliminary work it was found that brand new plastic petri dishes gave poor germination and growth even after being thoroughly washed and rinsed. This was presumably caused by toxic material leaching from the dish. It was found that this problem could be solved by soaking the dishes in plain seawater for at least two weeks. All glassware and petri dishes were washed thoroughly with a cleaning agent (Lipsol) followed by four rinses with tap water and two rinses in distilled water and then soaked in distilled water overnight to reduce the chances of any toxin remaining. Glassware was oven dried but plastic petri dishes were not, in case the raised temperature caused chemical breakdown and further release of toxic substances.

The use of plastic petri dishes has advantages over glass coverslips (often used in crystallising dishes). Firstly, it permits rapid change of growth medium without the danger of coverslips overlapping each other, thus disturbing tetraspores and sporelings. Secondly it permits direct non-destructive observation under a microscope to monitor the germination of tetraspores and growth of sporelings with minimal stress. The culture dishes were placed in a refrigerated waterbath (180 x 90 x 30cm) set at 10 ± 0.5^{0}C. This was illuminated from above with 2-8 1500mm green Thorn EMI fluorescent 65/80W tubes.

3.0. Photon irradiance

Green light was used exclusively throughout the studies, with irradiance ranging from 0.3 to 160 μmol $m^{-2}s^{-1}$. Green light was used because it was believed to reduce contamination by green algae (Holt,1983; Grandy,1984) supporting the idea of Kageyama and Yokohama (1977) that the photosynthetic efficiency of some green algae

is lower in green light than in white light [though this has been contested (Dring, 1981)]. Various irradiances were achieved by a combination of the number of fluorescent tubes used, and a reduction of the incident light with combination of black nylon net and chiffon attached to square wire frames (25 x 25cm) which fitted over a black wooden grid with squares of the same dimension. It is difficult to achieve exact irradiances especially when replicates are required, hence they were grouped together in irradiance bands. Each compartment of the grid contained four culture dishes. The positions of different irradiances were arranged according to the average photon irradiance on that particular grid.

Experiments on germination were carried out mainly at irradiances between 15 and 40 μmol $m^{-2}s^{-1}$, except in some experiments where the irradiance ranged from 10 to160 μmol $m^{-2}s^{-1}$. A light regime of 12:12 h (light:dark) was used.

For studies of growth of ***Palmaria*** sporelings two light regimes were used, 8:16 h and 16:8 h (light:dark). These ran concurrently in the same waterbath, shielded from each other by a black light-proof polythene screen. Initially light measurements were made with a Crump Quantum Radiometer Photometer CAT. NO550. Towards the end of the studies a more accurate light meter, a Licor model LI-1000 became available. The Crump light meter was later calibrated against the Licor light meter using quantum sensor serial NO:Q5232. It was found that the relationship between readings from the two light meter were linear with factor of 1.6. The original irradiances were converted by multiplying by a factor of 1.6.

4.0. Medium

Various growth media have been used by different workers in red algal culture. They are usually enriched seawater media such as Erdschreiber (Sparling, 1961; Boney and Corner, 1962), f/2 (Burns and Mathieson, 1972), SWM-3 (McLachlan and

Edelstein, 1977; van der Meer and Chen, 1979) and Provasoli's enriched seawater (ES) medium (West, 1967; Lee and West,1980; Lee and Kurogi, 1983).

Provasoli's ES medium has been used successfully in the past at the Port Erin Marine Laboratory for culturing various red algal species (Grandy, 1984). In the present study various growth media were tried out including Iwasaki's SW11, Grund (VS), f/2, SWM-3 and Provasoli's ES medium (Appendix 1). With the exception of Provasoli's media, all the other media used were full concentration. Since Provasoli's medium was widely used at Port Erin, full concentration and half concentration were used using filtered autoclaved seawater and plain filtered seawater. It has been shown that filtered seawater gave better growth for ***Alaria*** gametophytes than filtered autoclaved seawater (Walton, 1986). Seawater was filtered through Whatmans GF/C filter paper and then autoclaved for fifteen minutes at 103 kPa. It was left to cool until it reached experimental temperature, i.e 10^0C, and aerated vigorously before use to replenish the air driven out during autoclaving. Plain filtered seawater was obtained by filtering seawater through 0.45 micron Millipore filter paper. The nutrients were then added just before use.

For growth studies, particularly to test the effect of different growth media, seawater of the same batch was used throughout. The seawater was kept frozen and thawed when needed. To prevent cultures being contaminated with diatoms, 2 mg/l germanium dioxide (GeO_2) were added to the culture medium. Lewin (1966) first reported GeO_2 as a specific inhibitor of diatom growth and suggested that a concentration of 1-10 mg/l would be sufficient to suppress diatom growth while allowing other algae to grow normally. Since then GeO_2 has been widely used in culture work in many laboratories. It has been found that GeO_2 inhibits the growth of brown and green algae (McLachlan ***et al.***, 1971; Markham and Hagmeier, 1982) and short term exposures to concentrations of 0.045-0.175 mg/l GeO_2 are now recommended for these cultures (Markham and Hagmeier, 1982). However inhibitory effects from GeO_2 have not

been observed in cultures of red algae (Chen ***et al.***, 1970; Chen, 1977; McLachlan and Edelstein, 1977; Markham and Hagmeier, 1982; Grandy, 1984).

In germination studies, Provasoli's ES medium half concentration using autoclaved filtered seawater was used throughout. In some experiments the growth hormones indole-3-acetic acid (IAA) and kinetin, were added in concentrations ranging from 10^{-9} and 10^{-3}M to find out whether the addition of growth hormone would enhance the germination of tetraspores. A stock solution of IAA was prepared by dissolving IAA first in a small quantity of ethanol and adding distilled water to make up the volume. IAA of the required concentrations were obtained by diluting the stock solutions. Stock solutions of kinetin were prepared by dissolving kinetin first in dilute hydrochloric acid which was neutralised with dilute sodium hydroxide and adding distilled water to make up the volume. Kinetin of a required concentration was diluted from the stock solution. The growth medium was changed every seven days or when the sporelings were being measured. Each culture dish was filled with 100ml of growth medium.

5.0. Measurement and Calculation

To calculate the percentage of germination of tetraspores a count of between 250 and 280 tetraspores (dead and alive) were made six days after the time of release (Charnofsky ***et al.***, 1982; Burns and Mathieson, 1972). Based on several trial experiments it was found that tetraspores began to settle and germinate 1 to 5 days after release. Living or dead tetraspores were categorised by the presence or absence of pinkish red colour (Prince, 1973). Dead tetraspores lost red pigment and disintegrated. Damaged tetraspores still retaining the red pigment but surrounded with dark mucilage were also counted as dead. Percentage germination was calculated as follows:

$$G\% = \frac{\text{no of germinated tetraspores}}{\text{no of germinated and dead spores}} \times 100$$

where G% = percentage germination

There are several ways to measure the growth of young algal sporelings. The technique used may vary with species and with age. One method is to count the number of cells per plant (Boney and Corner, 1962; Burns and Mathieson, 1972) but in polystromatic algae this is suitable only during the first two or three weeks of development. Linear dimensions such as diameter when the plant is disc shaped or filament length where filaments are produced may also be used (Jones and Dent, 1971). With ***Palmaria*** sporelings, the shape changes from a disc shape when very young to a filament shape as it grows older. Quite often more than one filament is produced from a single plant and the shape of the filament varies considerably.

One of the best ways to monitor the growth of ***Palmaria*** sporelings is to measure the area, as it takes into account any changes in shape, which linear measurements do not. The disadvantage is that it does not include any changes in thallus thickness: in early growth many cell are produced with little change in area. However this is not very serious as in ***Palmaria*** growth take place mainly in two dimensions with relatively little thickening in the thallus. Although the flat blade of the sporeling has two sides, only the area of one side was measured. Observations of the sporelings were made ten days after inoculation and again after 31-35 days. Prince (1973) reported that after 8 to 12 days an erect shoot was differentiated from the central region on the upper surface of the holdfast. In Port Erin an erect shoot began to appear on days 10 to 12. After the tenth day it is very difficult to trace the area of attached sporelings properly without any distortion in the drawing. At the end of an experiment, that is after 31 days, germlings were carefully dislodged from the petri dish before tracings of their area were made (Plate 1.1).

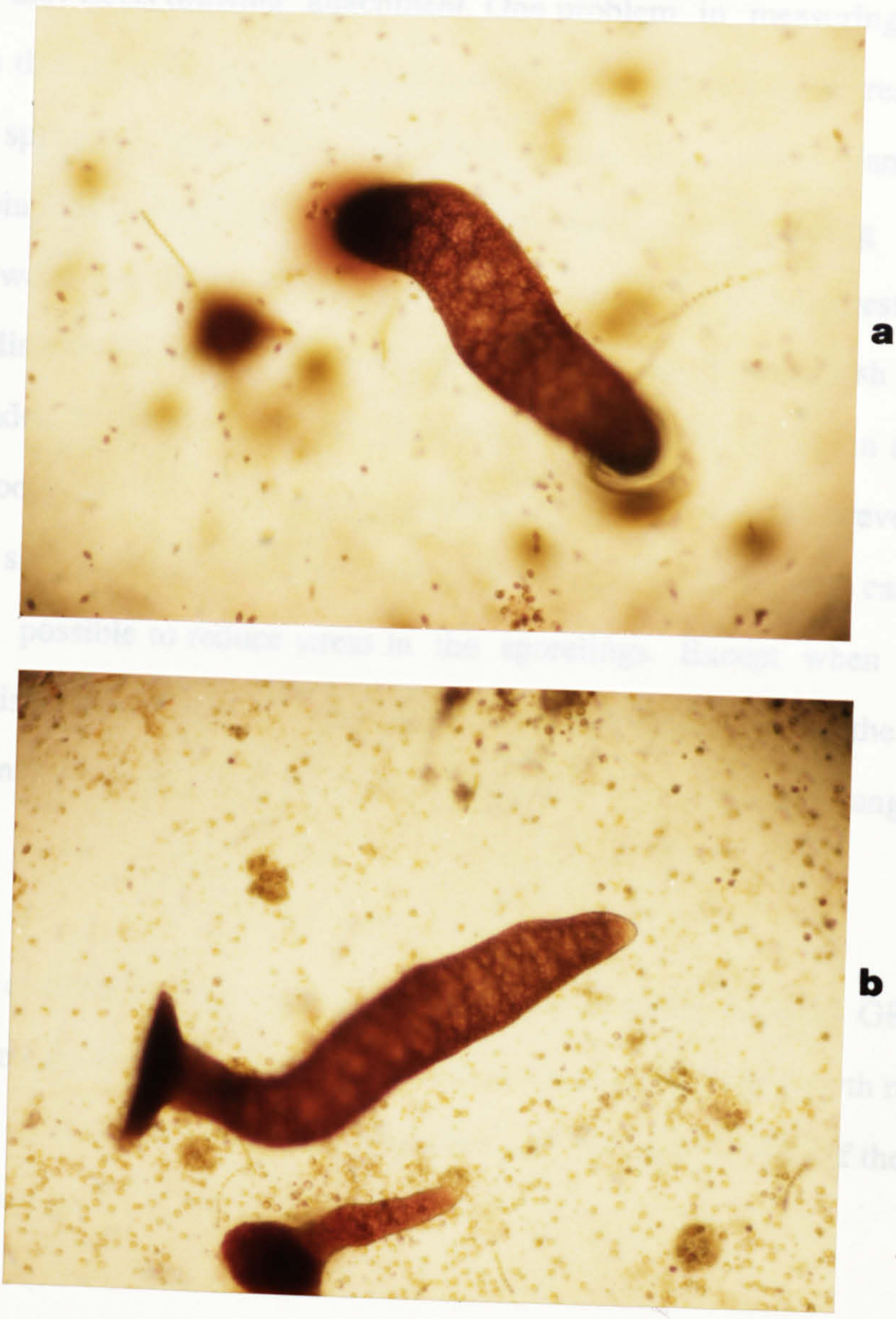


Plate 1.1. ***Palmaria*** sporelings 35 days old a) before and b) after being dislodged.

Each culture dish was examined in turn using an Olympus compound microscope fitted with a Carl Zeiss drawing attachment. One problem in measuring the area of sporelings is that not all tetraspores germinated at the same time, resulting in a variation of sporelings size within a particular culture dish. Stunted plants resulting from clumping of two or more sporelings and small plants arising from late germination were excluded. Where possible the area of 30 of the largest sporelings from each dish were drawn. This was done by placing a petri dish containing sporelings under the microscope. All observations were carried out in a constant temperature room at 10^0C, with growth medium in the petri dish to prevent it from drying and to slow down any increase in temperature. Observations were carried out as quickly as as possible to reduce stress in the sporelings. Except when sporelings have to be dislodged this is a non destructive method which enables the growth of individual plants or a group of plants to be followed without any change in plant density.

The plan area of the sporelings was measured using a sonic digitizer GRAF/BAR MK11 interfaced to BBC microcomputer Model B. From these, area growth rates could be calculated. Growth rate calculation was obtained by modification of the equation by Hunt (1978).

The mean relative growth rate per day for an individual plant can be calculated by:-

$$RT1\text{-}T2 = \frac{Ln\ A2 - Ln\ A1}{T2 - T1}$$

Where R = mean relative growth rate per day for individual plant

A1 = plant area at time T1(days)

A2 = plant area at time T2 (days)

Ln = log base 2

For a group of plants, mean relative growth rate per day was calculated by :-

$$RT1\text{-}T2 = \frac{\frac{Ln\ A2 - Ln\ A1}{n2 \quad n1}}{T2 - T1}$$

Where n1 = number of plants at time T1

n2 = number of plants at time T2

Results

1.1. Sporing materials

Palmaria plants appeared to remain healthy with deep red pigmentation in the outdoor conical tanks even without fertilization, from October to early April. A deep red colour in ***Palmaria*** plant indicates that nutrients are not limiting (Simpson ***et al.***, 1978b). Visual observation indicated that ***Palmaria*** plants continued to grow slowly during this period, based on thin apices with red/pink colour. The thallus gradually narrowed toward the apices. The growth was however not measured during this time.

At Port Erin in the Isle of Man, by late October and early November most of the plants in tanks became reproductive while at the same time very few plants in the sea were reproductive.

1.2. Methods of obtaining *Palmaria* spores

Results from Experiments 1 and 2 (Fig. 1.2) showed that filtered spores gave better germination than unfiltered spores. A Mann Whitney non parametric test (Zar, 1984) showed that filtered spores gave significantly better germination at $P< 0.01$ in the Experiment 1 and $P< 0.02$ in Experiment 2.

Spores that failed to settle but were still alive eventually died. During this study it was found that germination only took place once the spore had settled and attached on to substrate. Similar observations were made elsewhere on several red algae species (Chemin, 1937; Sparling, 1961; Chamberlain and Evans, 1973; Mshigeni, 1976). Spores that failed to attach eventually died.

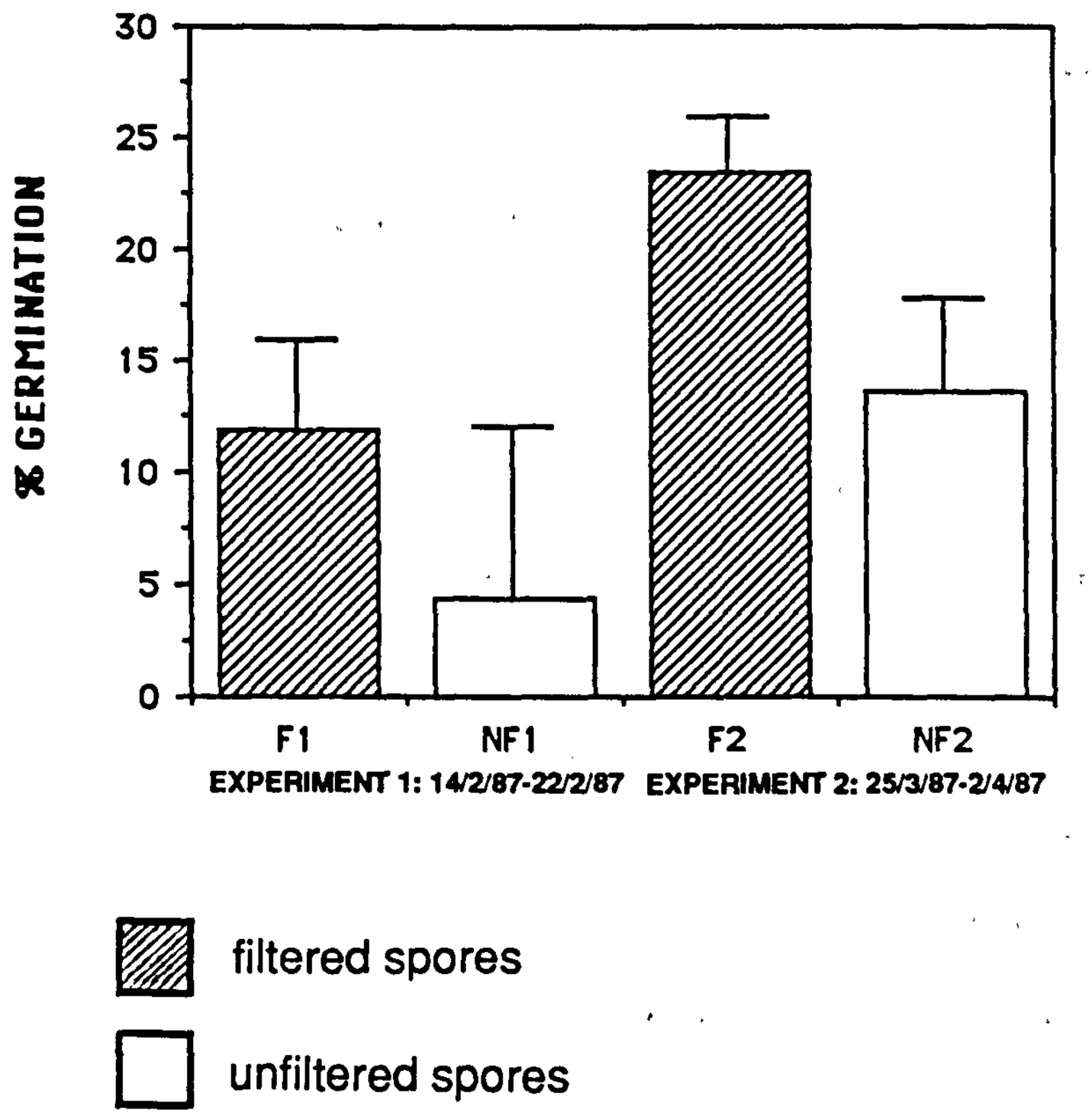


Fig 1.2. Percentage germination of filtered and unfiltered ***Palmaria*** spores with 95% confidence intervals.

Fig. 1.2 shows that viability of spores varies from experiment to experiment at different times. To overcome these inconsistancies, spore germination experiments were carried out twice or more over a period of time to get a better picture of the effect of the treatment. During this study no consistent monthly differences in viability of spores were observed throughout the sporing season.

1.3. Optimum conditions for the germination of *Palmaria* spores

1.3.1. The effect of photon irradiance on germination of *Palmaria* spores

Palmaria spores germinate over a wide range of photon irradiances up to 145-155 µmol $m^{-2}s^{-1}$ tested in this study as well as in the dark (Fig. 1.3). Percentage germination in the dark in most cases was significantly lower than in the light (Table 1.1). Spores germinated in the dark died 2-3 days later if not exposed to the light. Percentage germination in the light appeared "light saturated" at 16-30 µmol $m^{-2}s^{-1}$. There was no significance difference in percentage of germination between 8-15 or 131-155 µmol $m^{-2}s^{-1}$ and those germinated at irradiances between 16 and130 µmol $m^{-2}s^{-1}$. The best percentage germination achieved in this study was 40%.

1.3.2. Use of plant growth hormones to improve germination of *Palmaria* spores

The percentage germination of ***Palmaria*** tetraspores varied witht the concentrations of indole-3-acetic acid (IAA) and kinetin used. Treating with IAA at a concentration 10-3M gave mixed results (Fig. 1.4). Percentage germination was higher than the control in Experiment 4 but lower in Experiment 2 and 3. Non parametric multiple comparison of the control with the other groups (Zar, 1984) was employed after Kruskal-Wallis test (Table 1.2). Table 1.2 shows that the germination in IAA at concentrations of 10^{-7} to 10^{-4}M were significantly higher than the control in Experiment 3 and 4 and 10^{-6} to

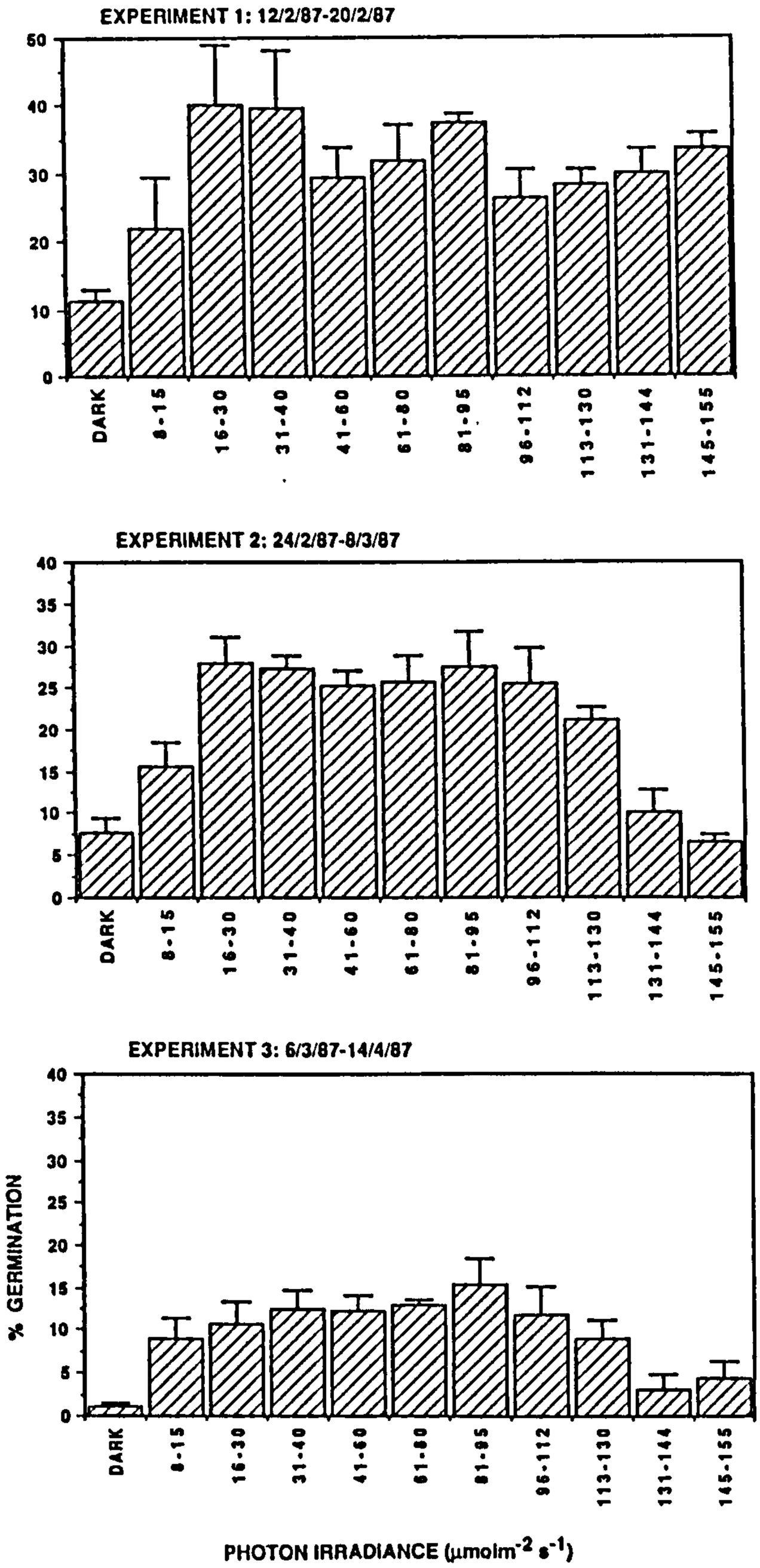


Fig. 1.3. Percentage germination of ***Palmaria*** spores in the dark and in different photon irradiances groups. Vertical bars indicate one standard deviation.

	Exp 1	Exp 2	Exp 3
Dark vs 8-15	++	++	++
Dark vs 16-30	+	++	++
Dark vs 31-40	+++	0	0
Dark vs 41-60	++	+++	+++
Dark vs 61-80	++	++	++
Dark vs 81-95	++	+	+
Dark vs 96-112	++	+++	+++
Dark vs 113-130	++	+++	++
Dark vs 131-144	++	+	++
Dark vs 145-155	++	0	++
8-15 vs 16-30	0	0	0
8-15 vs 31-40	0	0	0
131-144 vs 16-30	0	0	0
131-144 vs 31-40	0	0	0
145-155 vs 16-30	0	0	0
145-155 vs 31-40	0	0	0

+++= Significance at P<0.05
++ = Significance at P<0.1
+ = Significance at P<0.2
0 = no difference at P<0.2

Table 1.1. Mann-Whitney comparison of percentage germination in the dark and under various photon irradiances.

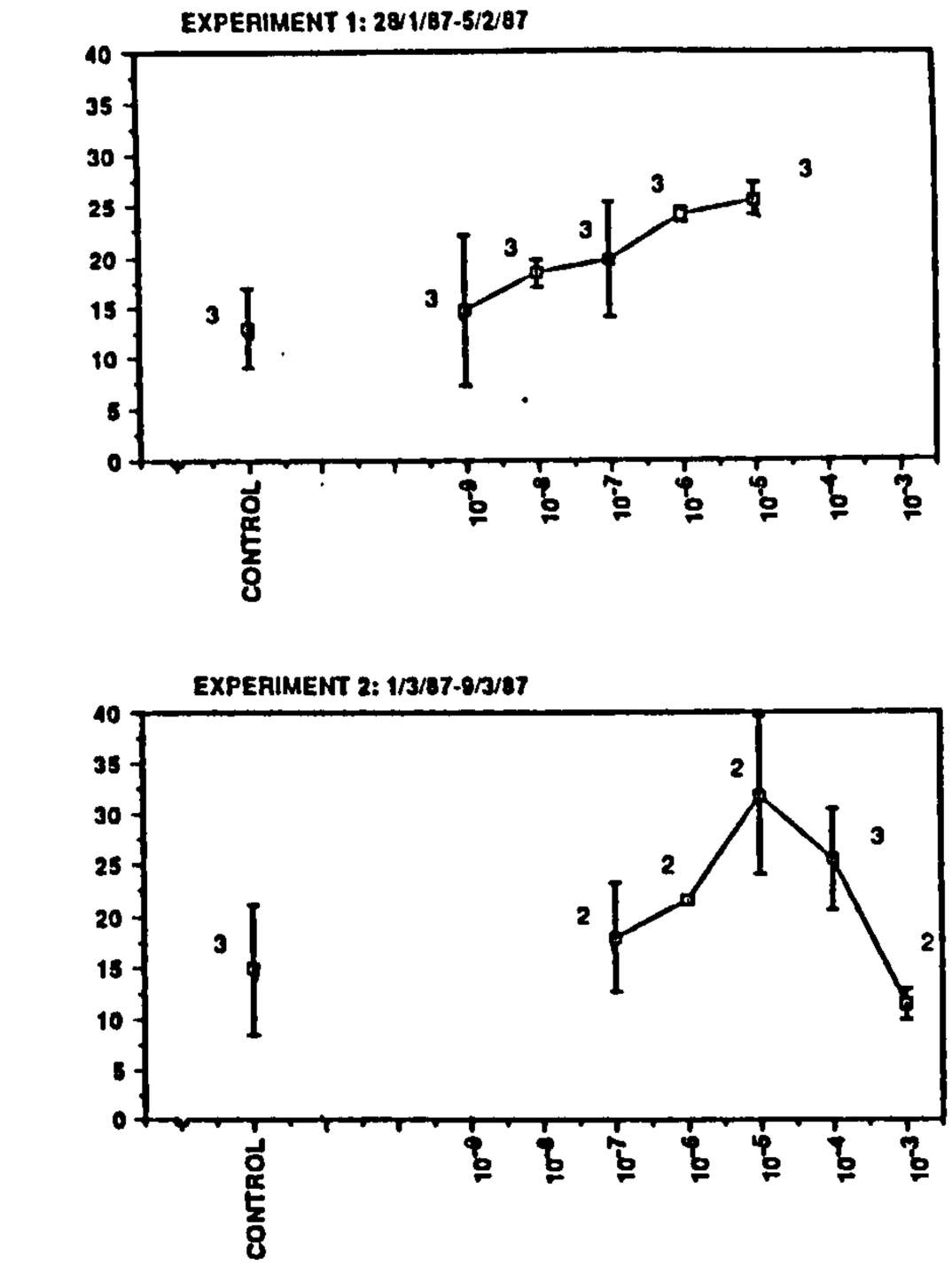


Fig. 1.4. Percentage germination of ***Palmaria*** spores treated with the plant growth hormone IAA at different concentrations. Vertical bars indicate one standard deviation. Number beside the vertical bar referrs to number of replicates.

10^{-5}M in Experiment 1. No Kruskal-Wallis was carried out in Experiment 2 because of the small sample size and high degree of variability between the samples. Better germination than the control was achieved in all experiments when IAA at concentration of 10^{-6} to10^{-4}M were used. IAA at a concentration of 10^{-6} to 10^{-5}M appeared to give maximum percentage germination in ***Palmaria*** spores.

IAA concentration versus Control	Experiment Number 1	3	4
10^{-9}M	0	-	-
10^{-8}M	0	-	-
10^{-7}M	0	+	+
10^{-6}M	+	+	+
10^{-5}M	+	+	+
10^{-4}M	-	+	+
10^{-3}M	-	0	0

(0) No difference at 5% significance level
(+) Significantly higher than control at 5% significance level
(-) No test carried out

Table 1.2. Non parametric comparison of a cont rol to other groups treated with various IAA concentrations.

Treating ***Palmaria*** spores with kinetin at various concentrations ranging from 10^{-9} to 10^{-3}M gave mixed results (Fig. 1.5). Non parametric comparison of the control with the other group (Zar, 1984) (Table 1.3) shows that the percentage germination was significantly lower than the control in Experiment 3 and 4 when kinetin at concentration 10^{-3}M was used. No comparison to a control was carried out in Experiment 2 because of small sample number and high degree of variability between samples.

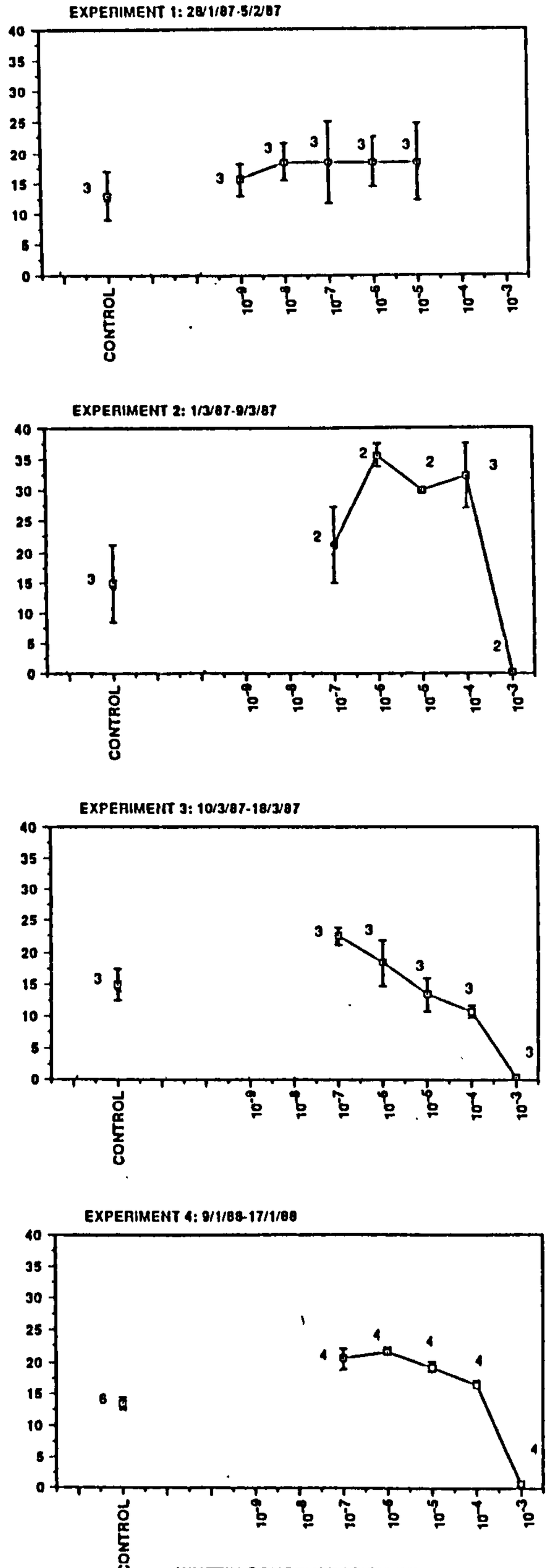


Fig. 1.5. Percentage germination of ***Palmaria*** spores treated with the plant growth hormone kinetin in different concentrations. Vertical bars indicate one standard deviation and the number beside the vertical bars reffers to number of replicates.

Kinetin concentration Versus Control	Experiment Number 1	3	4
10^{-9}M	0	-	-
10^{-8}M	0	-	-
10^{-7}M	0	*+	*+
10^{-6}M	0	0	*+
10^{-5}M	0	0	*+
10^{-4}M	-	*-	0
10^{-3}M	-	*-	*-

(0) No difference at 5% significance level

(*-/+) Difference at 5% significance level:

(*-= lower than control): (*+= higher than control)

(-) No test carried out

Table 1.3. Non parametric comparison of a control with other groups treated with various kinetin treatment.

Kinetin at a concentration of 10^{-4}M shows no difference in the percentage germination from the control in Experiment 4 but in Experiment 3 it was significantly lower. Kinetin at 10^{-9}M to 10^{-5}M produced no difference from the control in Experiment 1 but was significantly better in Experiment 3 and 4 at 10^{-7} and 10^{-7} to 10^{-5}M respectively.

The germination of the spores improved substantially as the concentration of kinetin was reduced to 10^{-4}M and lower. Kinetin at a concentration of 10^{-9} to 10^{-5}M in general gave higher germination than the control though not necessarily significantly higher than control. Kinetin at concentration 10^{-3}M seemed to be harmful to the spores as the percentage germination was very low.

Since the effects of kinetin treatment at various concentrations were different from one experiment to another, no conclusion can be made whether kinetin at certain

concentrations improves the germination of *Palmaria* spores. One thing which is clear from this study is that kinetin at 10^{-3}M seemed to be harmful to the spores.

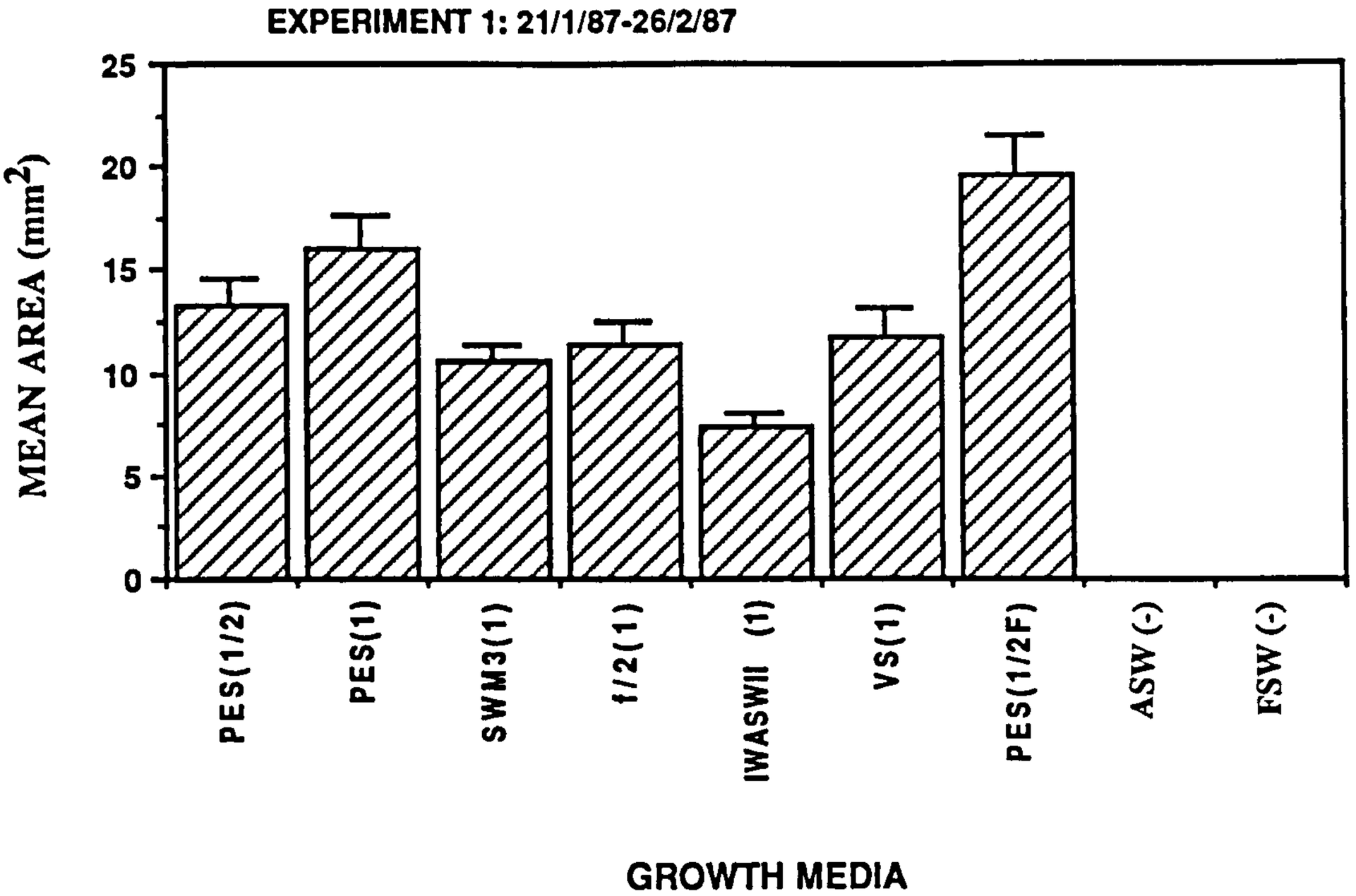
1.4. The optimum conditions for the growth of *Palmaria* sporelings

1.4.1. The effects of different growth media

Initially newly germinated sporelings grew well in all growth media tested as well as in plain autoclaved seawater and plain filtered seawater. In the second week most of the sporelings in both seawater died.

Results from the first experiment (Fig. 1.6) are shown by the mean area of *Palmaria* sporelings at 35 days old in various growth media. All the sporelings grown in plain filtered seawater and autoclaved seawater were dead. One way ANOVA test revealed that the growth of sporelings grown in various media were not equal at $P< 0.001$. Tukey multiple comparison (Zar, 1984) was employed to compare the growth of sporelings in different growth media (Fig. 1.7).

The mean area of *Palmaria* sporelings grown in Provasoli's media half concentration in filtered seawater ($PES^1/_2F$) was significantly higher than Provasoli's media full concentration (PES1) and half concentration ($PES^1/_2$), SWM3, f/2, Grund (VS) and Iwasaki's (SWII) in autoclaved seawater. There was no significant difference between PES1 and $PES^1/_2$. PES1 was significantly higher than VS, f/2, SWM3 and SWII. There was no significance difference between $PES^1/_2$ and VS, f/2 and SWM3 but the mean area of sporelings in all these media were significantly higher than in Iwasaki's SWII. There was no significant difference in area of sporelings grown in VS, f/2 and SWM3 but they were significantly higher than SWII. In order of magnitude, $PES^1/_2F$ had the highest mean area followed by PES1, $PES^1/_2$, VS, f/2, SWM3 and SWII had the lowest mean area of sporelings.



PES($^1/_2$) = Provasoli's ES medium half concentration in autoclaved seawater.

PES(1) = Provasoli's ES medium full concentration in autoclaved seawater.

SWM3(1) = SWM medium full concentration in autoclaved seawater.

f/2(1) = f/2 medium full concentration in autoclaved seawater.

IWASWII(1) = Iwasaki's SWII medium full concentration in autoclaved seawater.

VS(1) = Grund's medium full concentration in autoclaved seawater.

PES($^1/_2$F)=Provasoli's medium half concentration in filtered seawater.

ASW(-) = Plain autoclaved seawater.

FSW(-) = Plain filtered seawater.

Fig. 1.6. Mean area of ***Palmaria*** sporelings 35 days old in different growth media with 95% confidence interval. Media formulation as in McLachlan (1973) and Iwasaki (1961).

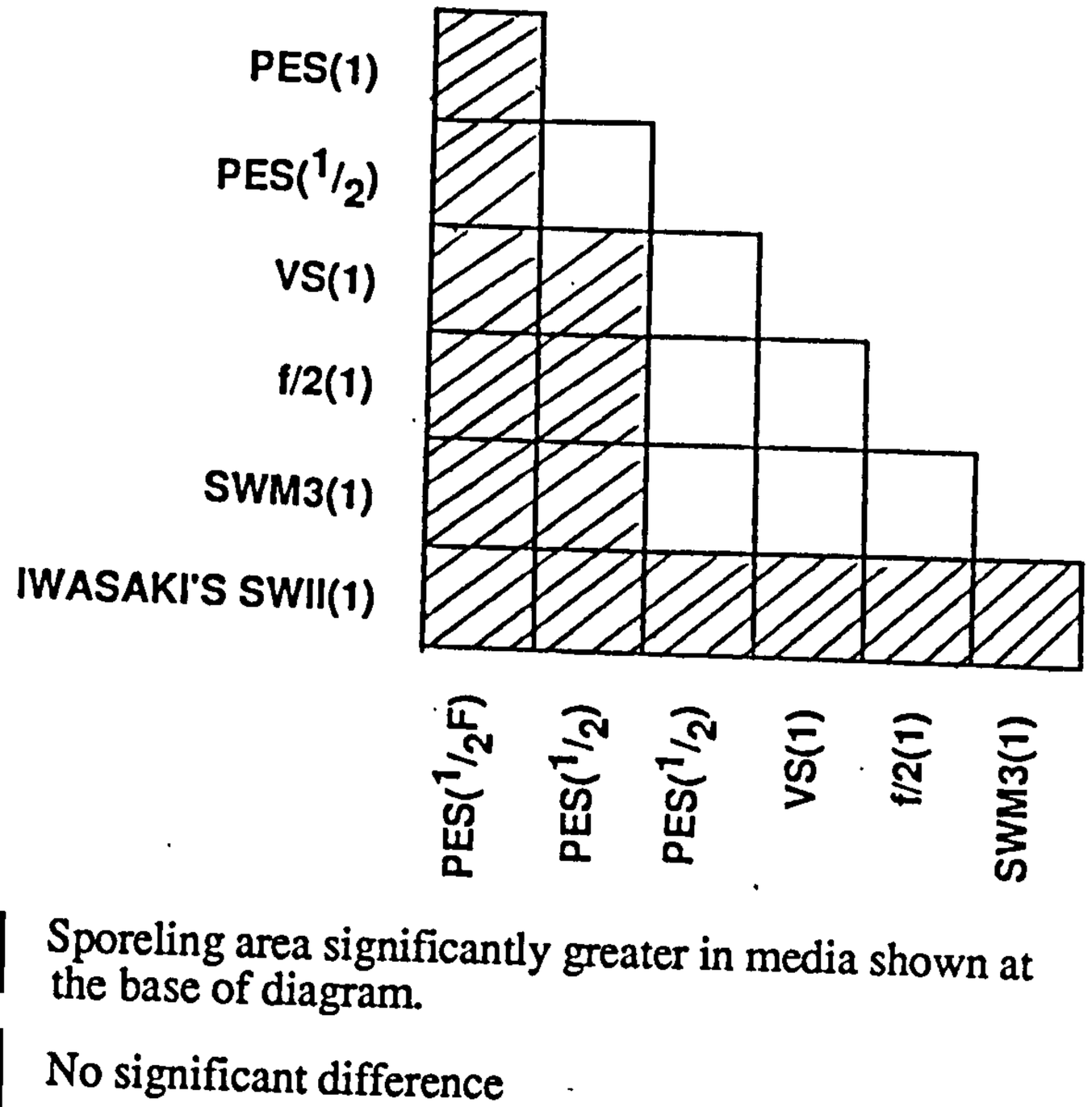


Fig. 1.7. Diagram showing a summary of a Tukey multiple comparison of the mean of ***Palmaria*** sporelings grown in different growth media. For key to media see fig. 1.6.

In Experiment 2, the mean relative growth rate of sporelings in term of area (R^A/Day) were measured from the 10th day and 24th day (Fig. 1.8) during which time the culture dishes were gradually contaminated with flagellates (Plate 1.2). Perhaps as a result there was a lag phase between the 24th and 31st day in all growth media (Fig.1.9). Contamination by flagellates in all growth media appeared to be the same though no quantative assessment was made. Although no statistical test was carried out, the results of Experiment 2 (Fig. 1.8) showed that PES$^1/_2$F supported the highest mean relative growth rate followed by PES$^1/_2$ and PES1, f/2, SWM3, VS and SWII in autoclaved seawater.

Sporelings measured on the 35th day (Fig. 1.10) showed that PES$^1/_2$F was the most successful medium followed closely by PES$^1/_2$, VS, SWM3, PES1, f/2 and SWII in autoclaved seawater even though all the culture dishes were contaminated.

1.4.2. The effect of photon irradiance and photoperiod on the growth of *Palmaria* sporelings

Initial experiments were carried out with 3 different photoperiods between the end of March and early May 1987. Two photoperiods, 8:16 h and 16:8 h (light:dark) were provided in two different constant temperature rooms set at 10^0C (air cooled) and another experiment was set up under continuous light in a water bath also at 10^0C.

Sporelings grown in photoperiods 8:16 h and 16:8 h (light:dark) were from the same spore suspension (3/4/1987) and the spore suspension for sporelings grown under continous light were obtained on (25/3/1987). Although the sporelings were grown at different times the seawater for growing sporelings was identical, that is collected on the same day, filtered to 0.45 micron, frozen and thawed when needed. Whenever possible a photon irradiance of 12-164 µmol $m^{-2}s^{-1}$ was used.

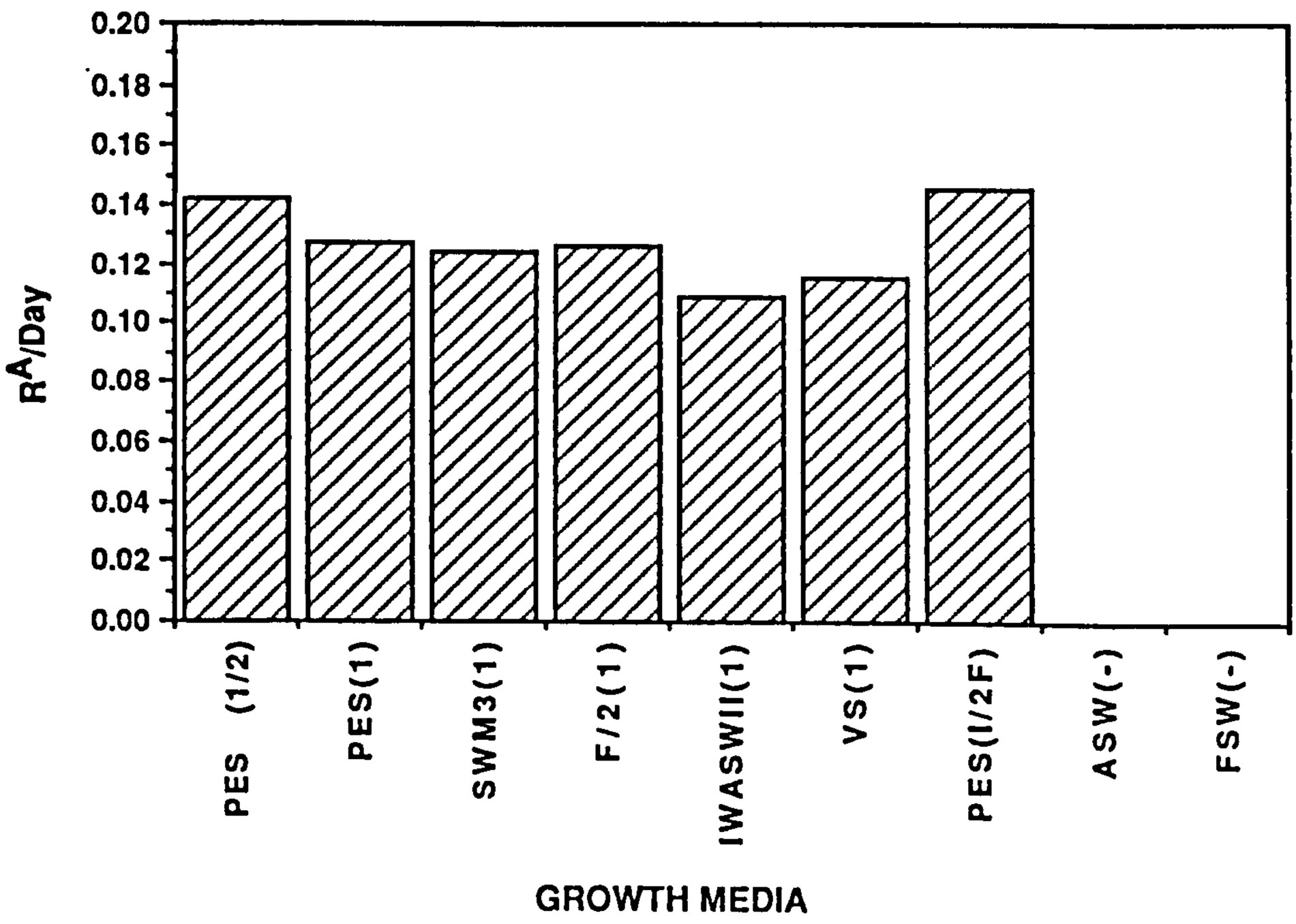


Fig. 1.8. Mean relative growth rate of ***Palmaria*** sporelings measured on the 10th and 24th days old in different growth media. For key to media see Fig.1.6.

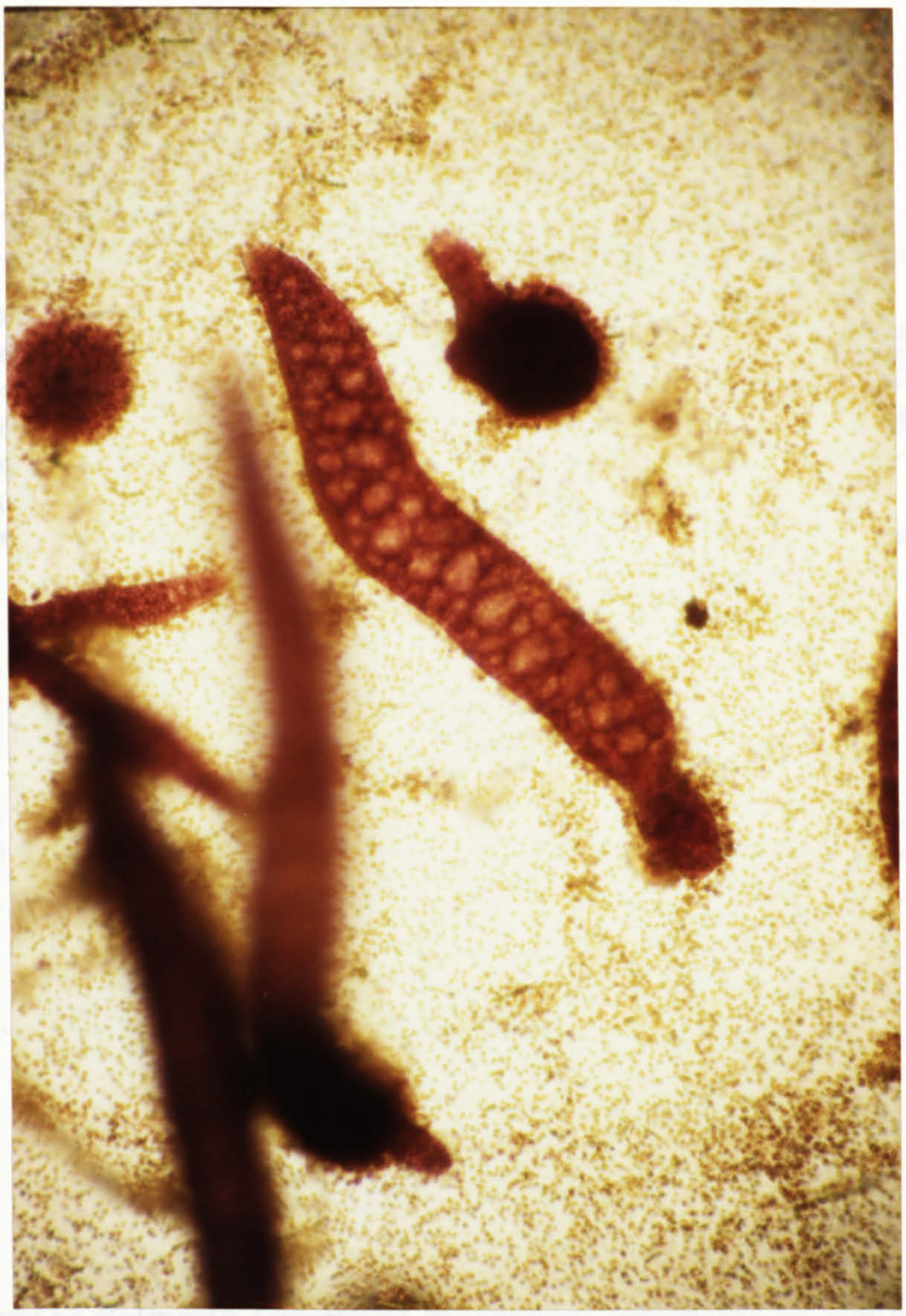


Plate 1.2. ***Palmaria*** sporelings 35 days old, contaminated with flagellates.

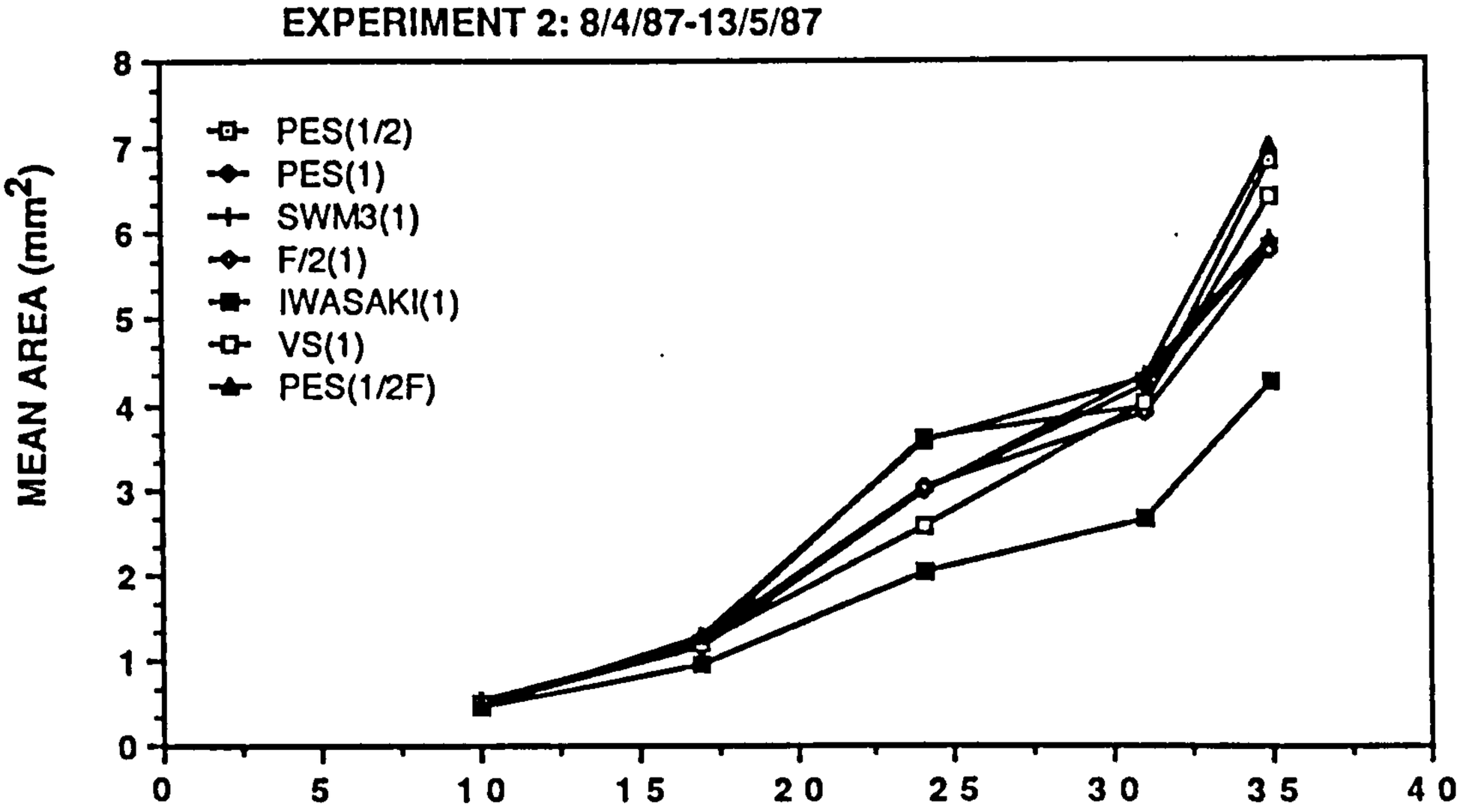


Fig. 1.9. Growth of ***Palmaria*** sporelings in different growth media. For key to media see Fig.1.6.

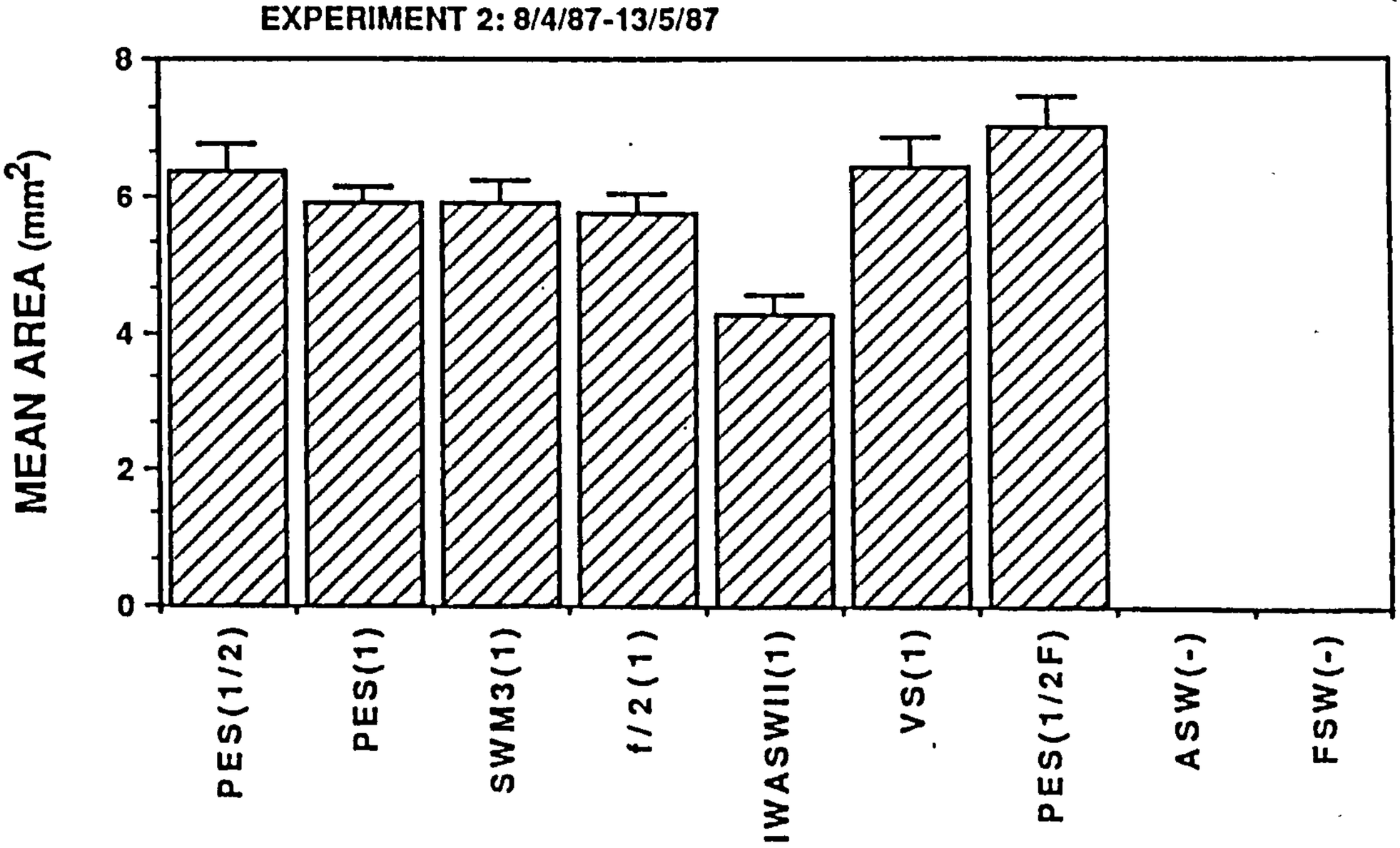
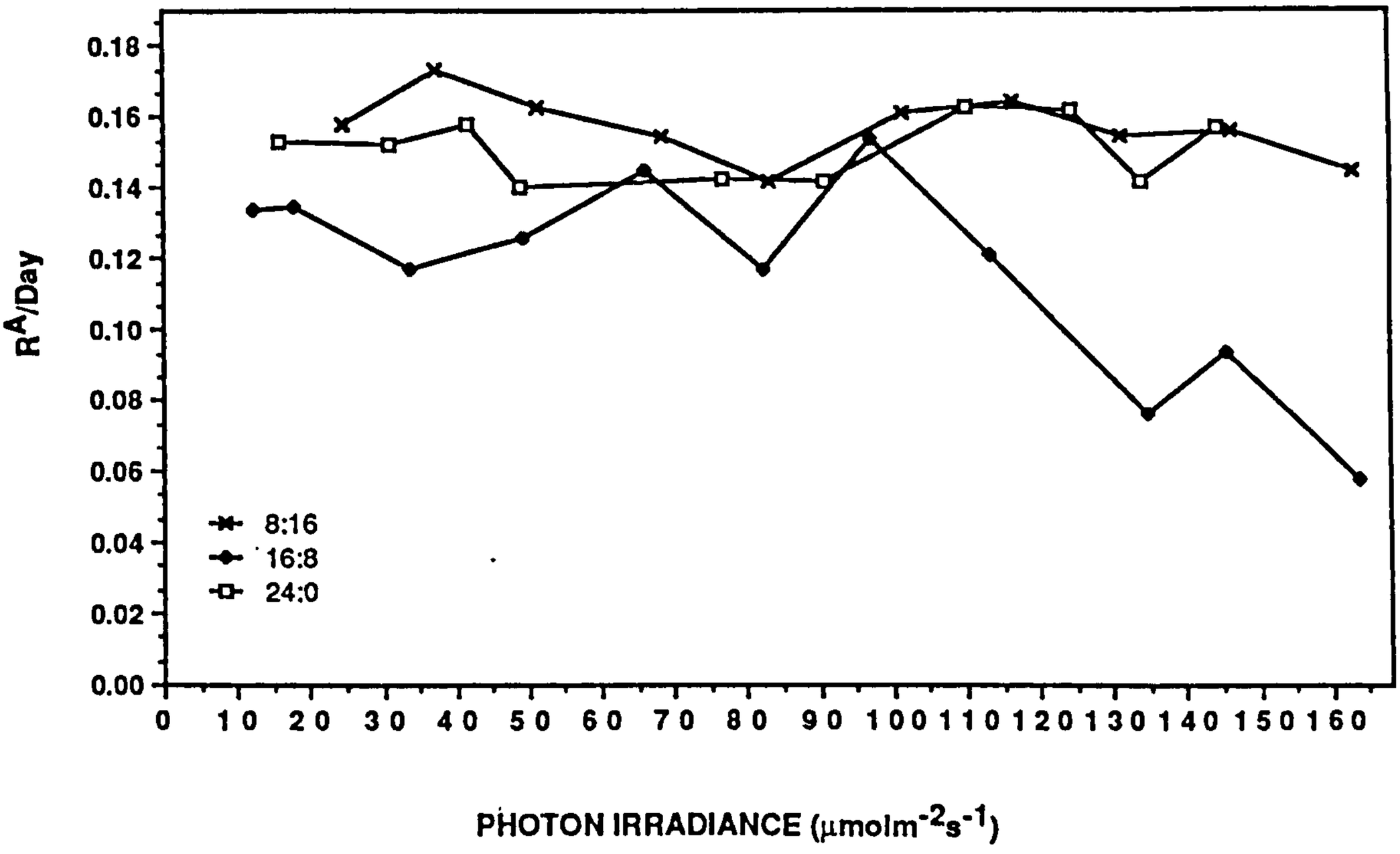


Fig. 1.10. Mean area of ***Palmaria*** sporelings 35 days old in different growth media with 95% confidence intervals. For key to media see Fig.1.6.

Results from this experiment (Fig. 1.11) shows that the mean relative growth rate fluctuated between 0.132 and 0.173 in 3 different light regimes. Results from 16:8 h photoperiod showed a lower growth rate than the other two light regimes and the growth rate dropped sharply above 97 μmol $m^{-2}s^{-1}$. Later it was discovered that the temperature control mechanism in this constant temperature room was faulty. The temperature fluctuated between 8 and 15^0C or more. Visual observation indicated that the extent of the contamination in the culture dishes in the 16:8 h were comparable to the other two light regimes, however the sporelings and filamentous green algae that contaminated the culture diswhes were pale in colour. Perhaps this could be the effect of high temperature.

The mean relative growth rate of sporelings grown under 8:16 h and continuous light shows random fluctuation over the whole range of photon irradiance. Varying levels of sporeling density and level of contamination in the culture dishes probably caused the fluctuation of growth rate and masked the effect of photon irradiance and photoperiods. A high level of sporeling density resulted in many sporeling clumping together, producing stunted growth (Plate 1.3) making it difficult to measure. Most of the contaminants consisted of unicellular and filamentous green algae. Of these two, filamentous green algae were considered more deleterious to ***Palmaria*** sporelings as they grew fast, occupied much space, may have competed for the available nutrients and blocked light from reaching the ***Palmaria*** sporelings. Sporelings grown in heavily contaminated culture dishes were stunted with a lot of hyaline hairs (Plate 1.4).

It is improper to compare statistically the growth rate of sporelings grown under 8:16 h and continous light since the sporelings were grown from spore suspensions collected at different times, there was a varying level of contamination (mentioned previously) and the culture conditions differed (i.e the 8:16 h group were grown in a constant temperature room and the continuous light group were grown in a water bath). The sporelings grown in the water bath under continuous light had more stable



8:16 h - 3/4/87-8/5/87
16:8 h - 3/4/87-9/5/87
] from same spore suspension

24:0 h - 25/3/87-29/4/87

Fig. 1.11. Mean relative growth rate of ***Palmaria*** sporelings in 3 different photoperiods: 8:16 h; 16:8 h; 24:0 h (light:dark).

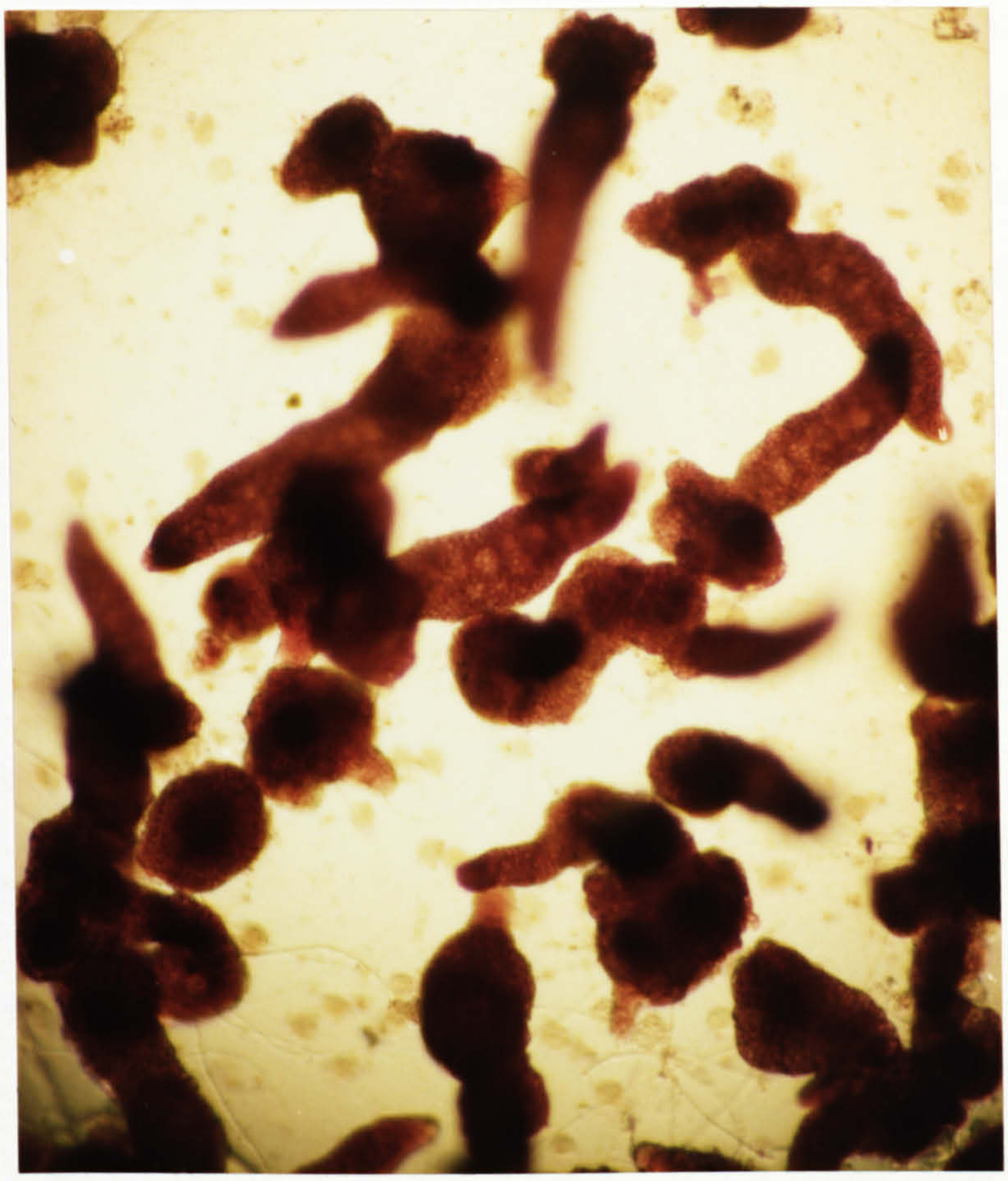


Plate 1.3. High density of ***Palmaria*** sporelings 35 days old in a culture dish shows stunted growth and unusual morphology due to clumping of 2 or more germinating spores.

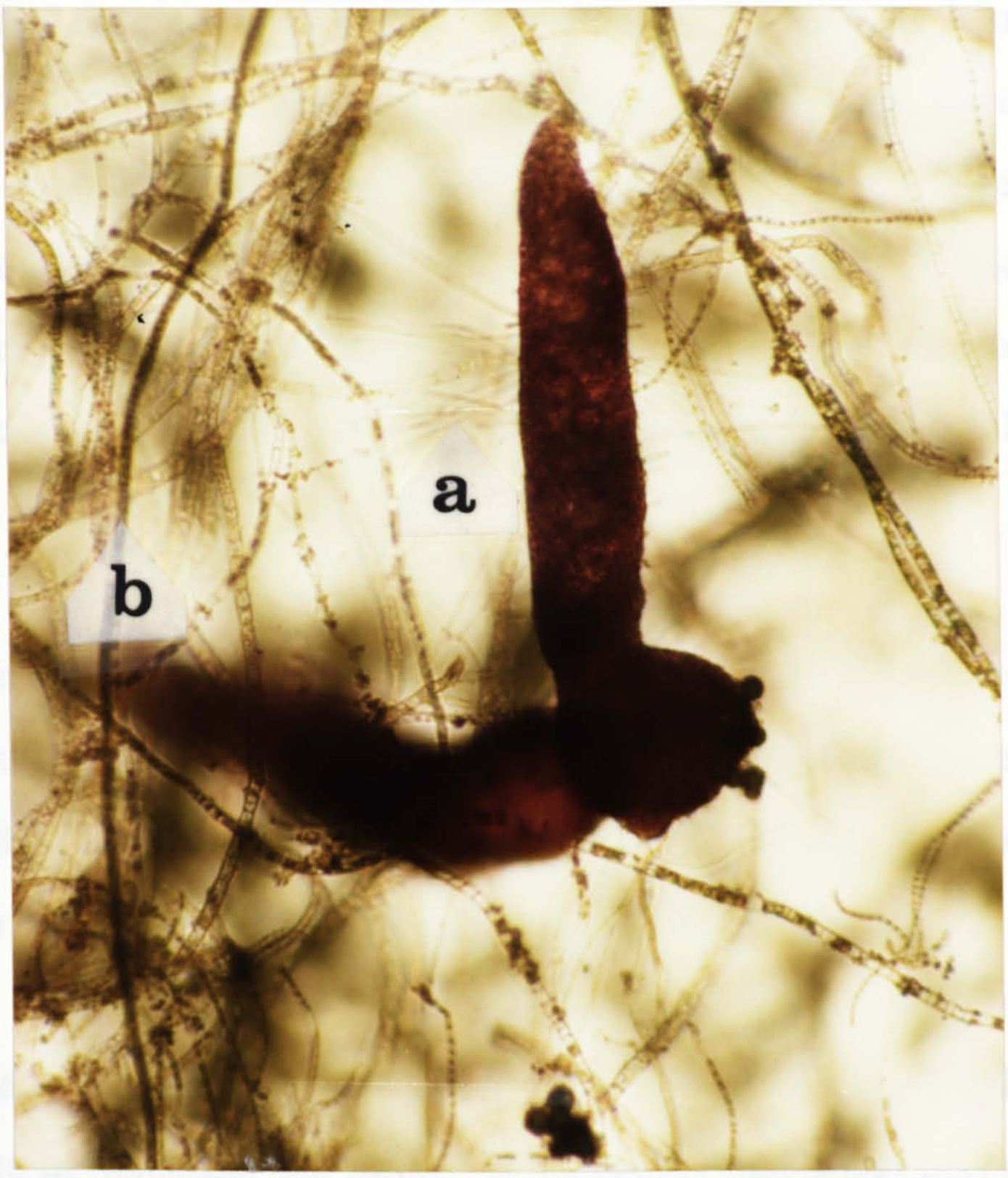


Plate 1.4. ***Palmaria*** sporeling in a heavily contaminated culture dish, a) hyaline hairs b) filamentous green algae.

temperature. Visual observation showed that the level of contamination in the culture dishes under continuous light were heavier than those under 8:16 h.

A second, scaled down experiment, was carried out in February to March 1988 to find out the effect of photoperiod and irradiance on ***Palmaria*** sporelings.Two photoperiods were chosen, 8:16 h and 16:8 h (light:dark) and the experiment was carried out in the same waterbath at 10^0C as described in Materials and Methods. Photon irradiances of 0.38-80 µmol $m^{-2}s^{-1}$ were used. Steps were taken to ensure that sporelings in each culture dish were of the same density, with very little or no contamination.

This time no major contamination was present in either photoperiods or under various irradiances. At the end of the experiment, that is after 31 days, all the sporelings grown at photon irradiance between 0.030 and 1.5 µmol $m^{-2}s^{-1}$ in both photoperiods were dead. Growth rates of sporelings grown at photon irradiances between 2.0 and 2.5 µmol $m^{-2}s^{-1}$ were very slow, 0.017 and 0.022 respectively, in both photoperiods (Fig.1.12). Under this irradiance, the germination was very low, the sporelings did not show much cell development and expansion. At photon irradiances beyond 2.5 µmol $m^{-2}s^{-1}$, the mean relative growth rate of sporelings increased with light in both light regimes. It appeared that saturation occured somewhere between 15 and 25 µmol $m^{-2}s^{-1}$. Because of wide range of irradiance used (0.3 to 80 µmol $m^{-2}s^{-1}$) and limited facility it was difficult to have enough replicates to grow at irradiance between 15 and 25 µmol $m^{-2}s^{-1}$. Sporelings grown under 16:8 h appeared to reach light saturation at 20.5 µmol $m^{-2}s^{-1}$ but it is not appropriate to say this for sporelings grown at 8:16 h because of the wide gap (11.4-26.7 µmol $m^{-2}s^{-1}$) between the replicates. The light saturation under 8:16 h probably lies between 15 and 25 µmol $m^{-2}s^{-1}$ mentioned earlier.

At photon irradiance beyond 2.5 µmol $m^{-2}s^{-1}$, the mean relative growth rate of

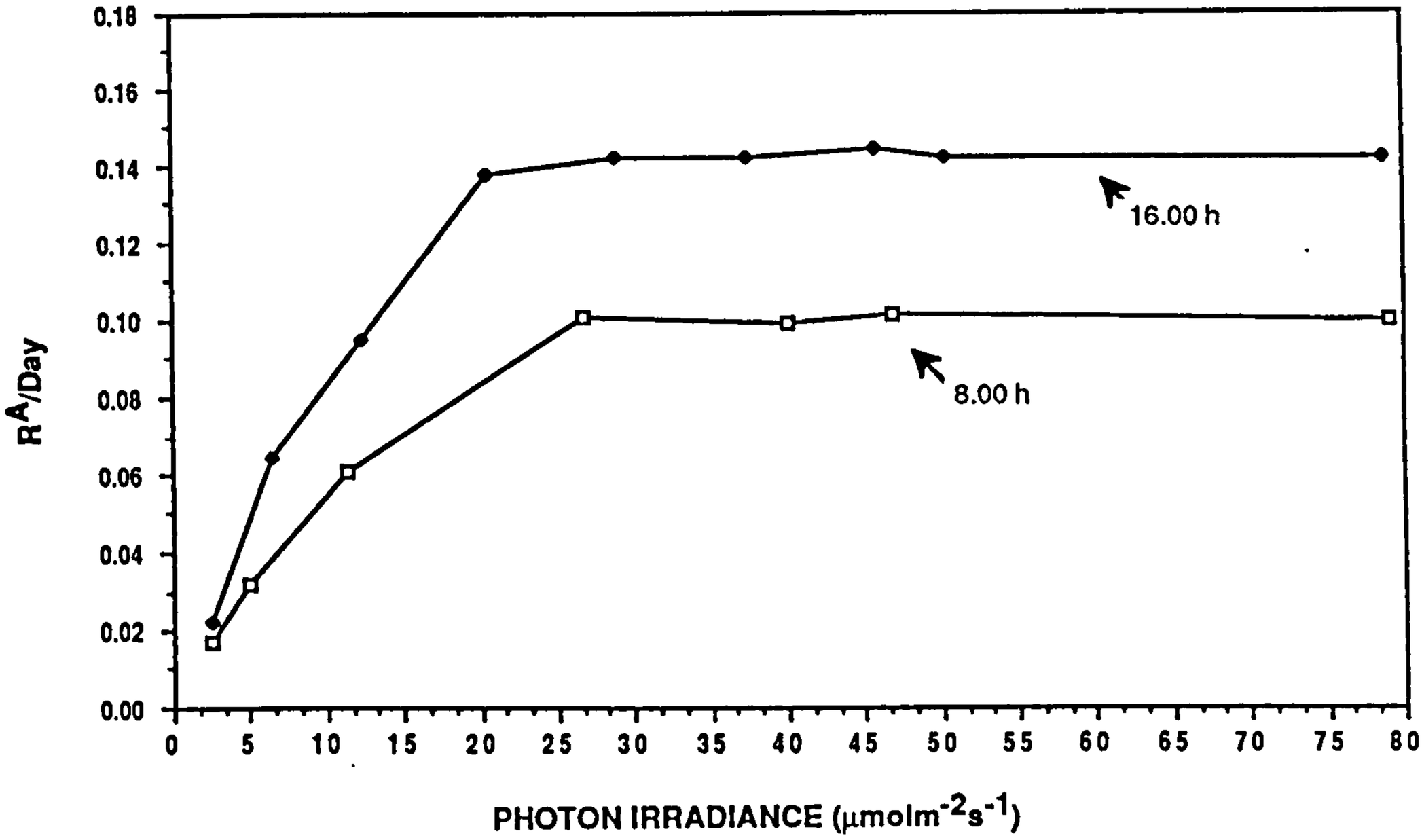


Fig. 1.12. Mean relative growth rate of ***Palmaria*** sporelings in 2 different photoperiods: 8:16 h and 16:8 h (light:dark) and under various photon irradiances.

sporelings grown under 16:8 h was higher than those grown under 8:16 h. The size and number of cells of sporelings grown in 16:8 h were greater than those grown in 8:16 h.

Discussion

Palmaria plants can be kept in a good condition during autumn to early spring without fertilization. The level of nitrogen and phosphate in the sea 5km off the west side of the Port Erin during this time was 2-5 µg at/l and 0.3-0.8 µg at/l respectively (Graziano, 1988), perhaps this is adequate to maintain the well being of the plants.

Palmaria plants kept in the outdoor tanks also became reproductive in late October and early November, 1-2 months earlier than in the field. The reproductive period for ***Palmaria*** on Port Erin breakwater generally occurs during winter and spring (Kain, 1982). A similar observation was made by Waaland (1976) who found that in tank cultures ***Iridaea*** plants usually reached reproductive maturity and produced spores or gametes about 1 to 2 month earlier than did the plants in the natural population from which they were originally collected. What caused the ***Palmaria*** plants in the outdoor tanks to reach maturity earlier than did the plants in the sea is still not known.

It is possible that low nitrogen and phosphorus levels during summer followed by a gradual increase of these nutrients during autumn provides a physiological cue for the induction of sporulation as has been reported by Knaggs (1967) in ***Rhodochorton floridulum***, Ramus (1969) in ***Pseudogloiophloea confusa*** and Oza (1977) in ***Falkenbergia rufolanosa.*** West (1972) stated that irradiance and temperature play a role in the formation of sporangia in the red alga ***Acrochaetium proskauri.***

Prince (1973) suggested that a number of factors may initiate reproduction, including a decrease in temperature, irradiance and photoperiod and an increase in nutrient concentration. Temperature, light and nutrients may operate collectively or individually to initiate reproduction.

At Port Erin, the irradiance, photoperiod and nutrients concentration experienced by the *Palmaria* plants in the outdoor tanks are quite similar to those in the field but the temperature fluctuations experienced by the plants in the tanks are far greater than those in the field. Because of the smaller volume, the temperature of the seawater in the outdoor tanks is a few degrees higher during the day and a few degrees lower at night whereas the sea temperature is fairly constant. Average sea temperatures during the month of October and November (1986-1987) in Port Erin bay were 12.2 and 10.7^{0}C respectively (Slinn in prep.). Possibly rapid change in temperature coinciding with a decrease in irradiance and an increase in the level of nutrients trigger the early reproduction of *Palmaria* plants. Van der Meer and Chen (1979) found that *Palmaria* plants becomes fertile if incubated at a low temperature (5-7^{0}C) with a short light period 8:16 h (light:dark).

The fact that *Palmaria* plants bear tetraspores earlier in the outdoor tanks was an advantage because seeding of tetraspores on the cords could be carried out sooner. The ready availability of sporing materials meant less dependence on sporing materials obtained from the sea.

It was shown that germination of *Palmaria* spores only took place once the spores had settled and attached on to a substrate. Spore mucilage has long been known to play an important role in the attachment of algal spores (Suto, 1950; Nakazawa, 1958; Matsumoto, 1959; Boney, 1966; Linskens, 1966; Moorjani and Jones, 1972; Charters *et al.*, 1972; Chamberlain and Evans, 1973). Two distinct mucilage layers are involved in spore liberation and spore sheath formation in *Palmaria* (Pueschel, 1979; Boney, 1981). Boney (1981) suggested that a separate mucilage production within the spore is involved in actual adhesion.

Prince (1973) found that in culture the presence of a substance associated with isolated tetraspores of *Palmaria* (presumably the mucilage involved in the spore

release) interfered with attachment of the spores to the glass slides. It is possible that gentle filtration removed the remnant of the first mucilage layer thus allowing a better chance for the mucilage secreted within the spore to adhere to the substrate. The second mucilage is tenaceous and able to resist physical damage (Boney, 1981), such as caused by filtration, may offer protection against bacterial attack (Chamberlain and Evans, 1973) and at the same time allow diffusion of substances into and out of the spores (Boney, 1975). It is most likely that the third mucilage produced within the spore diffuses through the second mucilage and adheres to solid substrata mainly by chemical bonding since the polished surface of a plastic petri dish offers no opportunity for mechanical attachment (Baier, 1970). The ability of the spores to adhere to the substratum will depend on the biological conditions of the spores, the characteristic of the substratum, the physical parameters such as light, water motion, temperature and the properties of the water (Charters *et al.*, 1972). Whether the production within the tetraspores of mucilage responsible for adhesion starts only when a suitable substratum is detected is a question which still remalins to be answered.

One of the main problems in studying ***Palmaria*** spore germination is the variation of spore viability. Although van der Meer and Chen (1979) reported that spore viability varied depending on from where the sporing materials were obtained. This is not true in the present work since all the sporing materials were obtained from the outdoor conical tanks. One possibility could be the method of inducing spore release. Desiccation could result in the release of "unripe" spores which failed to germinate thus resulting in lower germination. Another possibility could be the seawater itself. The seawater supply in the laboratory is sometimes contaminated with hydrogen sulphide (H_2S) released by dead bivalves and other decaying organic matter under anaerobic conditions inside the pipes. Although precautions were taken to ensure the water was free from H_2S by flushing the pipe for some time, perhaps the traces of H_2S remained could have have killed the spores. Hydrogen sulphide is known to be highly toxic to

living organisms (Stecher, 1968). The presence of H2S is suspected to kill ***Laminaria*** gametophytes (Dawes, pers. comm.).

Photon irradiance seemed to play some role in germination of ***Palmaria*** spores. It has been mentioned earlier that percentage germination was significantly lower in the dark. In the light increased irradiance beyond 8-15 μmol $m^{-2}s^{-1}$ will not significantly increase percentage germination. Similar phenomena have been observed in several species of red and brown algae (Chen and McLachlan, 1972; Terry and Moss, 1981; Charnofsky ***et al.***, 1982; Correa ***et al.***, 1985). It has been shown that photosynthesis is involved in the germination of ***Bangia atropurpurea*** monospores (Charnofsky ***et al.***, 1982) but it is still not known if this is true in ***Palmaria***. Spores of some brown algae have been reported to be able to germinate in the dark even though the percentage germination was somewhat lower (Cosson and Gayral, 1978; Terry and Moss, 1981; Chen and McLachlan, 1972). The ability of some ***Palmaria*** tetraspores to germinate in the dark and continue to survive 2 to 3 days later may indicate that it is able to mobilise reserve material to undergo the germination process. This study showed that photon irradiance between 16 and 30 μmol $m^{-2}s^{-1}$ was sufficient to give optimal percentage of germination.

The use of plant growth hormone IAA appeared to improve the germination of ***Palmaria*** spores when the concentration of 10^{-6} to 10^{-5}M was used. However this finding had to be treated with caution for two reasons. Firstly IAA did not dissolve very well in seawater resulting in the variations in dissolved IAA concentration in the seawater. Secondly the IAA stock solution contained a small quantity of ethanol to dissolve it. Since the subsequent IAA concentrations were prepared from the stock solutions, the concentration of ethanol at each IAA concentration also varies. The increase in growth of micro and macroalgae in the presence of IAA and alcohol have been dismissed by Conrad and Saltman (1962) on the account that alcohol probably caused such increase in growth. Street ***et al.***, (1958) found that in absence of

ethanol, IAA produced only 12% increase in cell number of ***Chlorella vulgaris***, whereas in the presence of 0.4ml/l of ethanol, there was a 150% enhancement of yield. Such effects, attributed merely to the non specific nutritional value of the alcohol, were noted both in cultures grown in the dark and in the light. Some species of algae such as ***Chlorella vulgaris*** are able to utilize organic carbon in the dark (Shihira and Krauss, 1965; Lewin and Lewin, 1960; Lewin, 1963) but none of the red seaweeds are able to grow in the dark on organic carbon (Droop, 1974; Fries, 1973). Since there were very few published works on the use of plant growth hormones particularly IAA with and without the presence of alcohol, the role of IAA or alcohol or a combination of both in spore germination remains to be investigated.

According to Stecher (1968), kinetin promotes cell division in plant tissues and is physiologically active at a very low concentration but only in the presence of IAA. However in some cases kinetin alone produces positive effects on seaweeds. Kinetin stimulates the growth of ***Conchocelis*** of ***Porphyra tenera*** in axenic culture (Iwasaki, 1965). In this study, there is not enough evidence to suggest that kinetin improved spore germination. In fact kinetin at high concentration (10^{-3}M) appeared to be harmful to ***Palmaria*** spores.

Further investigation is required in order to assess the effect of plant growth hormones, or a combination of growth hormones, at various concentrations, on germination and to establish the role of hormones in the germination process, if there is any.

Germinated sporelings survived in unenriched seawater for up to 2 weeks, after which almost all were dead. In winter the average nitrate level in the sea off Port Erin is between 6 and 7 µg at/l and phosphate is between 0.6 and 0.8 µg at/l (Slinn and Eastham, 1984). Therefore plain autoclaved and the plain filtered seawater should contain enough levels of nutrients to support growth but because lack of water

movement in the culture dish, the sporelings probably cannot absorb the nutrient sufficiently fast. Water movement is known to enhance nutrient uptake in seaweed (Neish and Knutson, 1977; Lindsay and Sounders, 1979; Guerin and Bird, 1987).

The size and growth rates of ***Palmaria*** sporelings were not the same when grown in the different growth media tested. The growth response of sporelings to different growth media also varied with the seawater collected at different times. Different size and growth rates can also be observed in enriched filtered and autoclaved seawater. In both experiments it was clear that Provasoli's medium at half strength in filtered seawater (PES$^1/_2$F) supported better growth than the rest of the media in autoclaved seawater even though the difference is not always significant. One possibility could be that autoclaving precipitated some of the salts, or decomposed or altered a "growth factor" in the seawater (Johnston, 1962).

Generally PES medium supported better growth than SWM3, VS, f/2, and Iwasaki's SW11 although the difference was not substantial. Iwasaki's SW11 medium showed poor growth compared with other growth media tested probably because it lacked vitamins and trace metals. Prince (1973) found that ***Palmaria*** sporelings grown in growth media minus trace metals showed poor growth. On the other hand VS medium also lacks vitamins and trace metals but contains manganese supported a better growth than SW11. Perhaps lack of the manganese in SW11 has something to do with the poor growth. Manganese, an essential plant nutrient (Rains, 1976) is almost certainly essential to seaweeds because of its importance in photosynthesis (DeBoer, 1981). In a long term batch culture study, the presence of mangenese increased the growth of ***Conchocelis*** of ***Porphyra tenera*** (Iwasaki, 1967).

If ***Palmaria*** were to be grown on cords from spores, probably the best growth medium would be PES$^1/_2$F. Faster growth means less time in incubation and seeded cords can be transplanted to the field sooner.

The growth rates of ***Palmaria*** sporelings were faster in the longer photoperiod than in the shorter photoperiod. Dring (1967) showed that the growth of ***Porphyra tenera conchocelis*** was highest in 16:8 h followed by 12:12 h and 8:12 h.

At a given irradiance the amount of light energy received by the sporelings grown in 16:8 h theoretically will be twice than the amount received in 8:16 h light regime. This effect can be seen in Fig. 1.12 below growth saturation. In 16:8 h (light:dark) photoperiod at photon irradiances 6.4 and 12.4 µmol $m^{-2}s^{-1}$ the mean relative growth rates were 0.0644 and 0.0947 respectively. In 8:16 h (light:dark) photoperiod but with about twice the photon irradiance in 16:8 h, i.e 11.4 and 26.7 µmol $m^{-2}s^{-1}$ the growth rates were comparable at 0.0609 and 0.1009 respectively. Beer and Levy (1983) noted that ***Gracilaria*** sp. grown at a higher irradiance exhibited a higher light saturation and compensation point. Larkum and Weyrauch (1977) showed that in the red alga ***Griffithsia monilis*** as irradiance increased the efficiencies of chlorophyll a in the absorption of light energy also increased. Bird ***et al.***, (1979) observed that at similar total daily irradiance, a longer period of illumination resulted in increased growth rate of seaweed. On the other hand phytoplankton and seaweeds grown at lower photon irradiance have more chlorophyll a than those grown at higher photon irradiance (Beer and Levy, 1983; Lapointe and Tenore, 1981; Beale and Appleman, 1971; Durbin, 1974; Ramus ***et al.***, 1976). Since the efficiency of chlorophyll a is higher under higher photon irradiance there is no need for the seaweed to increase its chlorophyll a level whereas it is necessary to increase the level of chlorophyll a at lower photon irradiance rate to achieve optimum growth.

Problems regarding unequal sporeling density and contamination by unicellular and filamentous green algae have been mentioned earlier. In culture studies, particularly when comparing two or more variables, it is very important that replicates in each variable have more or less uniform sporeling density. A high density of sporelings

will result in stunted growth and unusual morphology (Prince,1973). Sporelings grown in crowded conditions (high sporeling density) and in a heavily contaminated culture dishes with unicellular or filamentous green algae have a tendency to produce more hyaline hairs than in normal conditions. Production of unicellular hyaline hairs is not uncommon in ***Palmaria*** sporelings and several other seaweeds in culture (Kylin, 1917; Sparling, 1961; Prince, 1973; Guiry, 1976) particularly in nutrient deficient media (Kylin, 1917; Prince, 1973; Sinclair and Whitton, 1977; Rueness ***et al.***, 1987). Probably the function of these hyaline hairs is to increase surface area to facilitate nutrient uptake (Rosenvinge, 1911; Whitton and Harding, 1978).

There are two possibilities for the production of unicellular hairs. Firstly the uptake of nutrients in the stagnant conditions of the culture is not fast enough to satisfy the need of the growing sporeling. This can be achieved by increasing the surface area for nutrient uptake. Secondly the fast growing filamentous green algae depleted the nutrient in the growth media may encourage ***Palmaria*** sporelings to produce hairs to maximize the uptake of dwindling nutrient to sustain growth.

Experiments on growth studies carried out in 1987 and 1988 indicated that the contamination by unicellular and filamentous green algae is seasonal. Relatively little or no contamination was encountered in the experiment carried out during autumn and winter. Contamination was quite serious in the spring and summer. During spring and summer, the use of green light and low irradiance was not effective in controlling the contaminant. While the use of GeO_2 at 2 mg/l is very effective in controlling diatoms throughout the study period without any ill effect to the ***Palmaria*** sporelings. It is very difficult to control unicellular and filamentous green algae. The use of penicilin, dihydrostreptomycin and streptomycin has been suggested by Provasoli (1968). However the effects of these chemicals on ***Palmaria*** sporelings are not known. The best time to carry out a culture study seems to be in the autumn and

winter when contamination is minimal.

CHAPTER TWO

Cultivation of *Palmaria palmata* vegetatively in onshore tanks

Introduction

Onshore tank culture of seaweeds was initiated in response to the increased demand for seaweeds, declining resources and uncertainties and seasonality of the natural products. Onshore tank culture allows seaweeds to be studied easily in a more controlled environment and provides a means of evaluating requirements, tolerance ranges and potential productivity of a particular species prior to development of costly mariculture facilities (Hansen, 1980).

Easy access to seaweed in tanks instead of in the field makes this method popular amongst phycologists who wish to develop intensive land based culture systems. Over the years, a number of factors affecting seaweed growth in tanks have been studied: e.g irradiance, temperature, salinity (Bird ***et al.***, 1979; Hanisak, 1979a; Lapointe, 1981), nitrogen enrichment (DeBoer ***et al.***, 1978; Lapointe and Ryther, 1979; Ryther ***et al.***, 1981; Bird ***et al.***, 1981, 1982), pH control and addition of CO_2 (DeBusk and Ryther, 1984; Bidwell ***et al.***, 1985), agitation (Waaland, 1973; Parker, 1982; Guerin and Bird, 1987), flushing (Lapointe and Ryther, 1978, 1979) and plant density (Lapointe and Ryther, 1978; Lapointe and Tenore, 1981).

In tank culture aeration is very important in the health of seaweeds (Waaland, 1973; Neish and Knutson, 1977; Guerin and Bird, 1987). According to Hanisak and Ryther (1984) the beneficial effects of aeration on seaweeds can be attributed to the following factors: 1) it increases photosynthetic efficiency by rotating the seaweeds in such a way that they are able to maximize the absorbance of light rather than having a high degree of

self shading; 2) it increases nutrient uptake rates by reduc ing the diffusion boundary layer; 3) it increases the availability of metabolic gases (CO_2, O_2) both by reducing the boundary layer and by direct enhancement from the air line; and 4) it flushes out competing algal cells and spores, thereby also reducing the epiphytes.

Studies in the field and in tank culture have shown that some seaweeds are known to have nutrient reserves and are able to utilize them to support growth when external nutrients are depleted (Hanisak, 1979a; Lapointe and Ryther, 1979; Chapman and Craigie, 1977; Ryther ***et al.***, 1981). DeBusk ***et al.***, (1986) suggested that the ability of N starved ***Ulva lactuca*** to absorb quickly and to store nitrogen indicates that a continuous supply in the medium is not required for algal growth, but that the alga's requirement can be met by periodic soaking in a concentrated nutrient medium. The ability to store nitrogen is common in a number of red seaweeds (Waaland, 1976; Neish ***et al.***, 1977). Pulse feeding reduces problems associated with continual nutrient addition, facilitates nutrient management and is as efficient when applied in the light or dark (Ryther ***et al.***, 1981). Lapointe (1985) suggested that the frequency of nutrient pulses was more important than the concentration in regulating growth of ***Gracilaria tikvahiae.***

Palmaria palmata grows satisfactorily in tanks, with growth rates comparable to other seaweeds in culture (Morgan ***et al.*** 1980a; Morgan and simpson, 1981a,b; Waaland, 1977, Davis, 1980). However these results were achieved under different solar radiations, with different strains and other local conditions. If the mariculture of ***Palmaria*** is to be considered, basic information about its biology is very important. Therefore the aim of this study was to determine its growth potential and potential problems that might arise from tank cultivation in Port Erin, Isle of Man.

Materials and methods

Two types of tanks were used, circular with a conical base, and rectangular tanks. Each conical tank had a surface area of 0.7 m^2 and a volume of 404 litre while each rectangular tank had an area of 0.59 m^2 and a volume of 474 litre (Plate 2.1). The two rectangular tanks were placed inside a bigger rectangular tank) and surrounded with flowing seawater from the conical tanks to avoid rapid changes in temperature. The conical tanks had a continuous flow of unfiltered seawater with an 8 to 17 volume exchange per day. The rectangular tank had a 1 volume change every two days. Fine mesh plastic screen was fitted to the outlet duct of each tank to prevent seaweeds from being lost. The salinity of the seawater was 33-34$^0/_{00}$ (Slinn, and Eastham, 1984) and the seawater pH was not regulated.

The rectangular and conical tanks were aerated from the laboratory piped compressed air system. The aeration and water flow in each tank could be regulated independently.

Palmaria plants were collected from Port Erin breakwater and the nearby area. Plants were cleaned, separated and healthy plants free from epiphytes selected. Plants of various sizes and age groups were used in cultures.

Whenever possible the initial stocking density was 1.3 to 2.4 kg/m^2 for the conical tanks and 1.4 to 3.0 kg/m^2 for the rectangular tanks. Plants obtained from the breakwater were deep red in colour, an indication of a high nitrogen content (Simpson *et al.*, 1978a; Morgan *et al.*, 1980a). Plants were conditioned for two weeks without any nutrient fertilization before being used following Morgan *et al.*, (1980a).

Both conical and rectangular tanks were pulsed with 0.1mM $NaNO_3$ and 0.01 NaH_2PO_4 (Morgan *et al.*, 1980a). Fertilization was carried out every two days in the

Plate 2.1. Growing ***Palmaria*** vegetatively in outdoor tanks at Port Erin, Isle of Man

a) conical tanks (continuous flow system)

b) rectangular tanks (batch system).

night, water flow stopped for 6-8 h and resumed again the next morning (Bidwell *et al.*, 1985). In case of the rectangular tanks, fertilization took place during the night the water was due to be changed.

Every week plants in the tanks were removed, placed in a fine meshed nylon bag and spun manually for about 2 minutes before weighing to the nearest gram. Calculation of relative growth rate were based on the formula put forward by Evans (1972).

$$R' = \frac{LnX1 - LnX0}{t}$$

Where R′= Relative growth rate per day

(R^A = Relative growth rate in term of area);

(R^W= Relative growth in term of weight)

X0= initial weight ; Wt= final weight ; t= duration of culture

Whenever necessary simple linear regression and ANOVA tests for significance of slopes were used to correlate growth, stocking density and hours of sunshine received. Daily sunshine records were obtained from Ronaldsway Meteorological Office, Isle of Man.

Results

Palmaria plants grown in conical tanks (continuous flow system) and rectangular tanks (batch system) remained healthy at first with a normal deep red colour similar to that in the natural population. But after a few weeks in culture the colour of the plants became dull and they were covered with epiphytes. The dull red colour was caused by fouling epiphytes which grew on ***Palmaria*** thalli not because lack of nutrients. The fouling by epiphytes became serious particularly in low plant density.

In 1986, the initial stocking density for each tank was not regulated to find out at which plants density will give optimum growth rate. Fig. 2.1a,b shows the results from the conical tanks (continuous flow systems). Results varied from tank to tank as the initial stocking density in each tank was not the same (Appendix 2) and there were other factors which will be discussed later. The highest relative growth rate in fresh weight per day (R^W) achieved for the conical tanks was 0.0430 and the lowest was 0.0027. The average R^W for the conical tanks was 0.0235.

Growth rates in the rectangular tanks (batch system) were lower than in the conical tanks (continuous flow system). The highest R^W was 0.0245, the lowest was 0.0020 and the average R^W in the rectangular tank was 0.0126. The average R^W for the batch system was about half that of the continuous flow system.

Results of the relationship between initial stocking density and relative growth rate, R^W are shown in table 2.1. The R^W in the conical tank No. 1 and rectangular tank No. 2 was inversely correlated to the initial stocking density at $P<0.05$ with coefficient of determination $r^2=0.29$ and 0.31 respectively (Fig. 2.2a,b).

Fig. 2.2a,b shows that R^W tends to fluctuate randomly as stocking density increases because of other factors which influence R^W such as sunshine, flow rate, state of

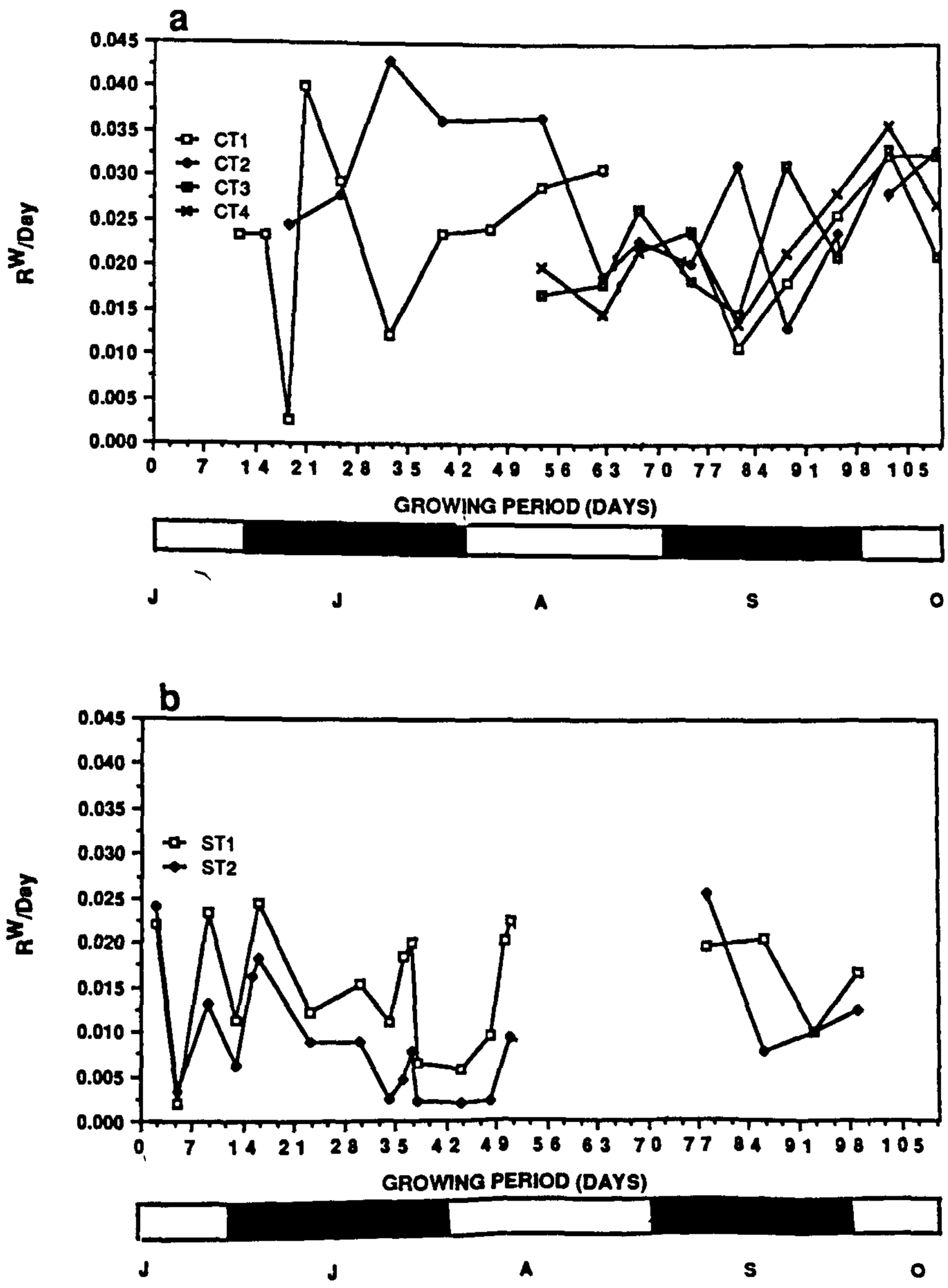


Fig. 2.1. ***Palmaria*** grown vegetatively in outdoor tanks in 1986,

a) conical tank (CT) in continuous flow system,

b) rectangular tank (ST) with batch system.

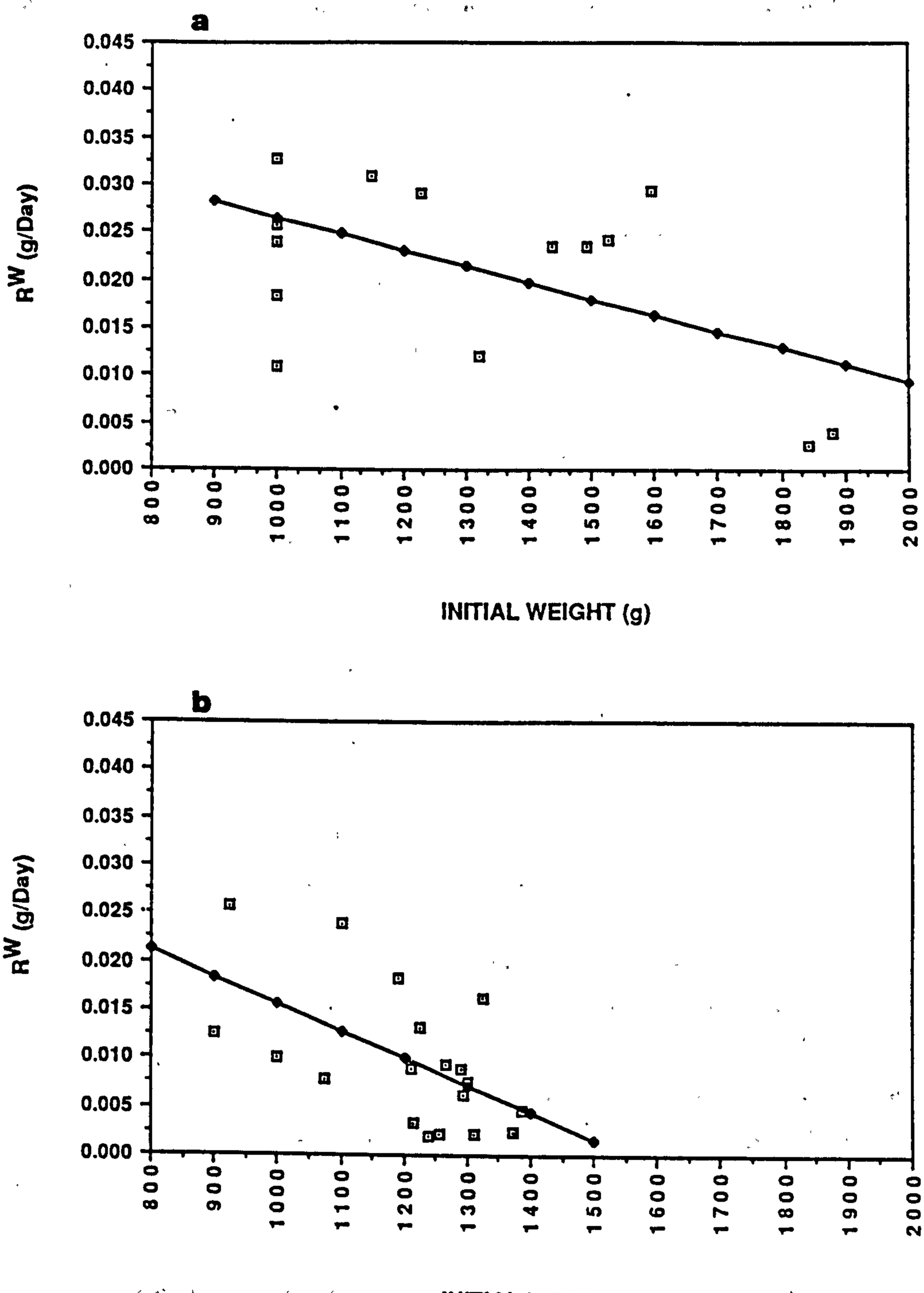


Fig. 2.2. Relative growth rate of ***Palmaria*** versus initial weight grown in outdoor tanks in 1986,

a) conical tank No.1 (continuous flow system),

b) rectangular tank No. 2 (batch system).

seaweed suspension etc. were not constant. The factors mentioned earlier probably were the reasons why there were no relationship between initial stocking density and R^W in the other tanks.

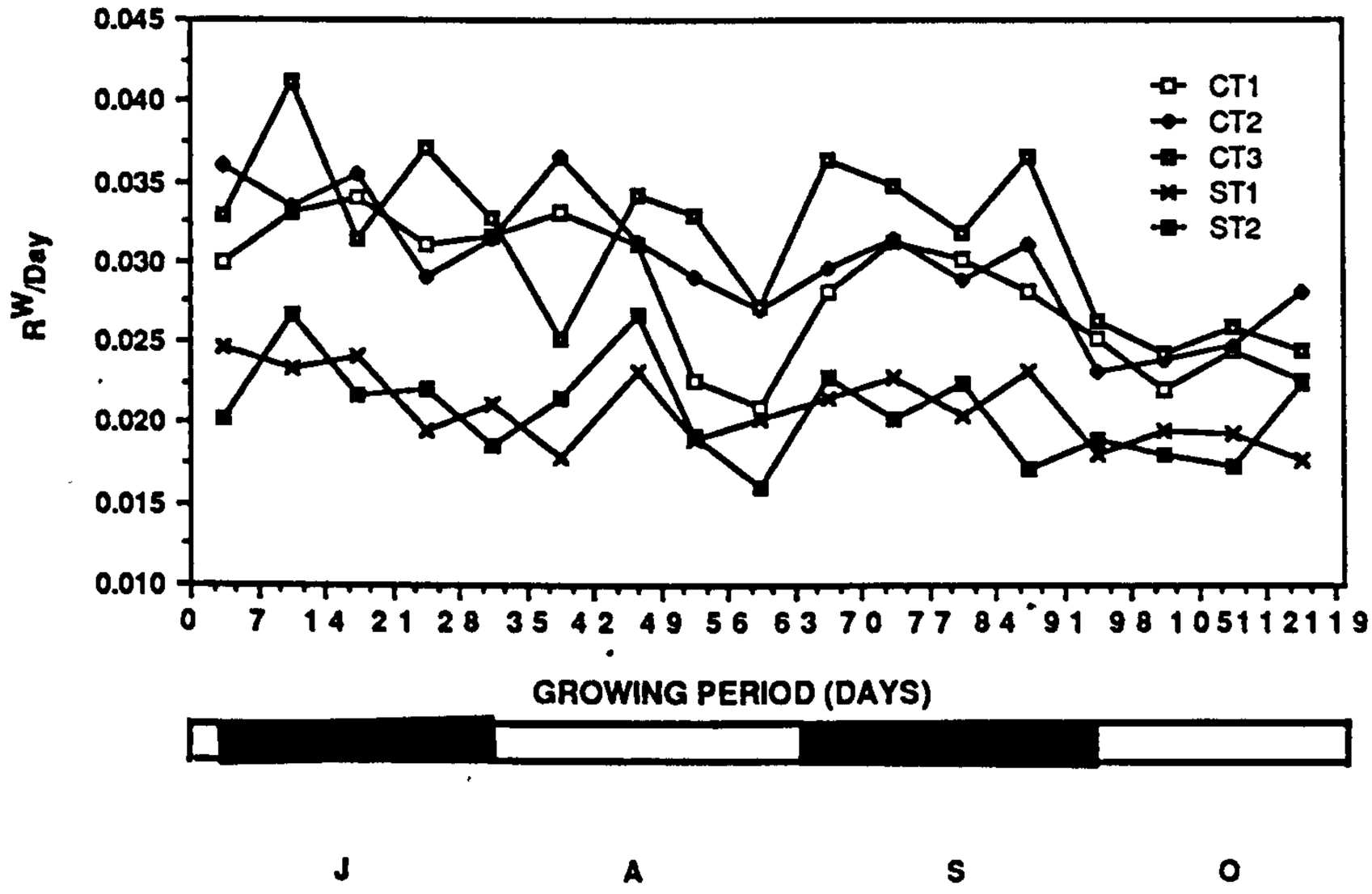
Tanks	Coefficient of determination r^2	ANOVA test P<0.05
conical tank No.1	0.29	+ -
conical tank No.2	0.08	-
conical tank No.3	0.01	-
conical tank No.4	0.15	-
rectangular tank No.1	0.03	-
rectangular tank No.2	0.31	+

+ R^W inversely correlated to the initial stocking density.
- no significant correlation between initial stocking density and R^W.

Table 2.1. Coefficient of determination and ANOVA test showing the significance of correlation between initial stocking density and R^W of ***Palmaria palmata*** grown in outdoor tanks in 1986.

A second experiment was carried out in 1987. The initial stocking density was kept constant at 1.3 kg/m^2 for the conical tanks and 1.44 kg/m^2 for the rectangular tanks. With the same plant density, R^W still varied from tank to tank (Fig. 2.3).

The highest R^W in the conical tanks was 0.0411 and the lowest was 0.0210. The mean R^W for the conical tanks was 0.0299. The highest R^W for the rectangular tank



CT1= conical tank number 1.

CT2= conical tank number 2.

CT3= conical tank number 3.

ST1= rectangular tank number 1.

ST2= rectangular tank number 2.

Fig. 2.3. Relative growth rate of ***Palmaria*** grown in conical and rectangular outdoor tanks in 1987.

was 0.0228 and the lowest was 0.0020 (Appendix 3). The mean R^W for the rectangular tanks was 0.0208 which is about one third lower than that of the conical tanks. Although the stocking density in 1987 was kept constant, the R^W was not significantly correlated at $P<0.05$ with the hours of sunshine received in either conical or rectangular tanks (Fig. 2.4a,b, Table 2.2). Again the effect of daily sunshine on R was being masked by other factors mentioned previously.

Tanks	Coefficient of determination r^2	ANOVA test $P<0.05$
conical tank No.1	0.17	-
conical tank No.2	0.07	-
conical tank No.3	0.12	-
rectangular tank No1	0.01	-
rectangular tank No.2	0.09	-

- no significance correlation between hours of sunshine and R^W.

Table 2.2. Coefficient of determination and ANOVA test showing the significance of correlation between hours of sunshine and R^W of ***Palmaria palmata*** grown in outdoor tanks in 1987.

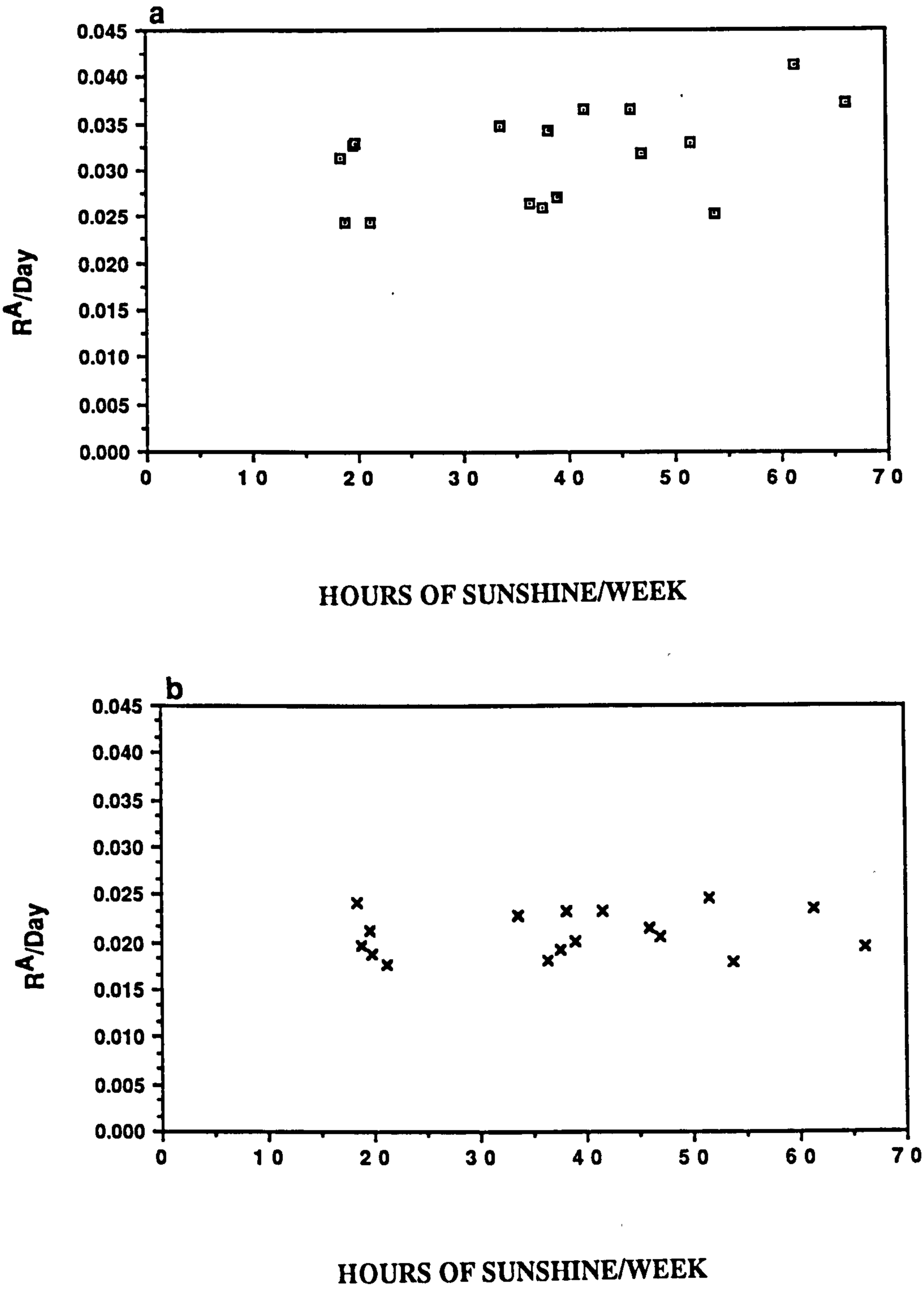


Fig. 2.4. Relative growth rate of *Palmaria* versus hours of sunshines grown in outdoor tanks in 1987,

a) conical tank No. 3 (continuous flow system),

b) rectangular tank No. 1 (batch system).

Discussion

Results from the outdoor tanks have to be interpreted with caution because of a number of factors involved which interacted with each other and reflect the overall growth of ***Palmaria.*** The factors involved are dynamic and it is difficult to isolate the effects of an individual factor unless experiments are carried out under more controlled conditions.

Results of experiments carried out in 1986 revealed that there was an inverse relationship between relative growth rate (R^W) and stocking density in conical tank No.1 and rectangular tank No. 2. As the initial stocking density increased, the R^W decreased though sometimes the relationship is not obvious because of influence by other factors which will be discussed later. The results obtained from this study agree with the results obtained elsewhere on ***Palmaria*** and other seaweeds by Simpson ***et. al.***, (1978a), Morgan ***et. al.***, (1980a) and Lapointe and Tenore, (1981). This is probably because at low density, most of the plants received enough light for optimum growth but as plant density increased, the plant did not get enough light due to self shading (Ryther ***et al.***, 1978) and movement of plants was restricted, they tended to clump together and thus thorough mixing was impeded (Simpson ***et al.***, 1978b).

In 1987 when the stocking density was kept constant the relationship between growth rates and hours of sunshine received was not very obvious, presumably because of the influence by several factors.

One of the reasons why there was no strong correlation between R and hours of sunshine received is because it did not take into account the total solar radiation. Smoke, mist, cloud cover, time of the year which affects sun altitude may vary the solar radiation hourly, daily, monthly and yearly. It has been shown that the relative

growth rate of seaweeds correlates with the amount of solar radiation received (Davis, 1980; Bidwell ***et al.***, 1984; Lapointe and Duke, 1984).

One of the factors which was probably responsible for masking the effect of stocking density and hours of sunshine received on growth rates is seawater flow rates. In the flow through systems used, the amount of water flowing through each tank was variable even though efforts were made to ensure that each tank had the same flow rate. Variation in flow rates are known to have an effect on R^W of seaweeds (Morgan ***et al.***, 1980a; Hanisak and Ryther, 1984; BeBusk ***et al.***, 1986). A blockage of some kind to any of the inflow pipes will reduce the water flow to that particular tank. There were times when the water flow in all culture tanks had to be reduced substantially because of the breakdown in the main seawater pump.

Another factor which is common to both flow through and batch systems is the state of seaweed suspension. Keeping the plant in suspension simulates the natural conditions in which tidal current and wave action maintain exchange of water around the plant surface (Waaland, 1973), enhances nutrient and dissolved carbon uptake and constantly cycles the seaweed in and out of sunlight (Guerin and Bird, 1987). Waaland (1976) observed that the amount of light received by an individual plant will depend on its residence time at the lighted surface where it receives full irradiances, at intermediate depths in the culture tank as it circulate about and at depths below the compensation irradiance. The pattern of water motion in these tanks is variable and an individual plant does not necessarily follow the same path on each cycle around the tank.

Achieving a good seaweed suspension is a common problem in flow through and batch systems. The state of seaweed suspension can be affected mainly by flowing seawater, aeration, size of plant and surface wind. Good suspension can be achieved by seawater flow from the bottom of the tanks, by aeration or by a combination of

both. Suspension achieved by seawater alone uses a lot of water and can be very expensive. Suspension achieved by aeration alone, due to poor tank design or poor positioning of the air line in the tank, sometimes develops a 'pocket' where there is very little water movement.

As a result, there is a tendency for the plants to aggregate in places where there is less water movement with no seaweed in the other areas to utilize the light energy. The situation deteriorates as plants get bigger. It is more likely that these plants become tangled and remain at the bottom because of their weight. A combination of aeration and water flow rate may help to reduce the formation of 'pockets' and improve seaweed suspension. A surface wind can also upset the state of seaweed suspension causing them to aggregate in one corner of the tank.

Results from cultivating ***Palmaria*** in onshore tanks for 2 consecutive years showed that the growth rates in the flow through systems were higher than in the batch culture. There are several possible reasons why the R^W of ***Palmaria*** grown in the flow through system were consistently higher than those in batch system even though the fertilization frequency and rates were similar.

According to DeBusk and Ryther (1984) the environmental conditions which occur in outdoor tanks receiving little or no water flow may be quite harsh relative to the requirement of macroalgal cultures. Temperature extremes, nutrient depletion and build up of toxic metabolites are among several detrimental conditions more likely to develop in batch culture than in flow through culture.

Plants grown in the batch culture appeared as healthy as those grown in flow through system; it is not likely that nutrients were limiting in batch culture since the seaweed was fertilized every two days and ***Palmaria*** is known to store nutrients as mentioned earlier.

Lapointe and Ryther (1978) observed that the growth of ***Gracilaria*** declined as the seaweed retention time increased even when the nitrogen and phosphorus loading were held constant. Lapointe and Ryther (1979) suggested that the daytime pH elevations in ***Gracilaria*** culture tanks may have been responsible for the reduced yields observed at low seawater exchanges.

Since the pH in the tanks was not measured it is not known whether the elevation of seawater pH during the day caused the slow growth in the batch culture. An increase in pH during photosynthesis is common in the tank culture of seaweeds (Simpson ***et al.***, 1978b; Morgan ***et al.***, 1980a). Braud (1984) stated that even with continuous seawater inflow the pH can reach values of 9.5 under high incident energy. At high pH the free CO_2 is almost completely unavailable resulting in substantial decrease in photosynthetic rate of the seaweed (Blinks, 1963; Ryther and DeBusk, 1982; Hanisak and Ryther, 1984). When light and nutrients are not limiting the growth of seaweed may be limited by the availability of CO_2 (Lapointe and Ryther, 1979; Hanisak and Ryther, 1984; Braud, 1984).

Although aeration provides a small amount of CO_2 in the seawater the amount is not enough to support the high growth of ***Palmaria***. Preliminary experiments carried out by Hanisak and Ryther (1984) showed that total inorganic carbon increased from 0.4 to 1.1mM after 9 days of aeration whereas the typical total inorganic carbon values in seawater are about 2.4mM.

Another possibility for the reduced growth in the batch system could be lack of micronutrients and accumulation of metabolic waste which is yet to be proved. Some red macroalgae seldom grow satisfactory in a batch culture system (Jones and Dent, 1970).

The relative growth rate of ***Palmaria*** plants grown in the flow through and batch systems in this study were consistently lower than those obtained by Morgan and Simpson (1981a,b) (0.059 and 0.077). Although the algal strain is different, the latitude (therefore solar radiations) differs, as do other environmental variables. However the explanation probably lies in scale for the high relative growth rates achieved by Morgan and Simpson (1981a,b) were with plants in an 8 litre tank where culture conditions could be easily managed. Furthermore only small plants or apical segments were used. The initial size and age of a plant has a large influence on the relative growth rate. In a big outdoor tank culture it is very difficult to obtain enough inoculum of small plants or apical segments. As a result plants of different size and age were used. Variation in size and age also arose because periodically some plants from each tank were harvested because of their size or fouling by epiphytes. The growth is faster when the plant is small and gradually declines as the plant becomes larger (Morgan ***et al.***, 1980a). This is also shown in Chapter Three where additionally, it is demostrated that relative growth rate of apical segments is almost twice that of the whole plants.

The fouling by epiphytes is a common problem in tank culture of seaweeds particularly in low plant density (Lapointe and Ryther, 1978; Morgan ***et al.***, 1980a; Yoneshigue-Braga and Baeta Neves, 1981). Epiphytes not only compete for nutrients in the tank, they also have disagreeable appearance and taste (Morgan ***et al.***, 1980a) thus lowering the market value of dulse.

It appears that pulsing the nutrient is not very effective in controlling epiphytes. Perhaps the epiphytes have the ability to store nutrients as efficiently as ***Palmaria.*** Controlling the epiphytes manually is very laborious, time consuming and totally unacceptable in the developed countries where labour is expensive. Increasing the plant density may help to reduce this problem (Morgan ***et al.***, 1980a) but it also reduces the growth rate of the cultured ***Palmaria.*** The present solution is a compromise between

seaweed quality and yield.

It has been mentioned earlier that optimum growth rates and yield can be achieved if factors that affect growth rates are kept at optimum levels but unfortunately some of these factors cannot be controlled economically, in particular solar radiation and temperature. Ultimately the success of commercial culture depends on solar radiation (Bidwell ***et al.***, 1984).

The potential of ***Palmaria palmata*** in the outdoor tank culture must not be judged on high growth rates and yield alone but other factors such as cost and practicality. First consider the cost of growing ***Palmaria*** in the outdoor tank. The cost includes infrastructure set up such as tanks, plumbing, pumps for seawater and air etc., operating cost which include running the pumps for seawater and air, fertilizer (nitrogen, phosphate, CO_2, maintaining pH) and manpower for maintaining the system. In Port Erin, Isle of Man where the cost of electricity is expensive, the bulk of the operating cost are likely to be for running pumps for seawater and air (Huguenin, 1976; Jackson, 1977; Guerin and Bird, 1987). Table 2.3 shows the estimated electricity costs of producing 1kg dry weight of ***Palmaria*** in an outdoor conical tank (continuous flow system) with 17 volume water change per day.

Cost of pumping seawater and air for one conical tank per week at 17 volume water change per day	= £0.445
Average yield per conical tank per week (in 1988)	= 233.8g
Dry weight yield per week (80% moisture)	=46.76g
Estimated cost of producing 1kg dry weight of ***Palmaria***	= £9.521

Table 2.3. Estimated electricity cost of producing 1kg dry weight of ***Palmaria*** grown in an outdoor conical tank excluding cost of drying and labour.

Demand for seawater can be reduced by controlling the pH, addition of CO_2 (Simpson ***et al.***, 1978b; Morgan ***et al.***, 1980a; Bidwell, 1984) and employing pulse fertilization. There is also the possibility that the need for aeration can be reduced by elimination of night time aeration (Bidwell ***et al.***, 1985; DeBusk ***et al.***, 1986). Fast growth rates can be achieved by growing small plants or growing apical segments (Morgan ***et al.***, 1980a; Morgan and Simpson, 1981a,b) but it is impracticable to do this on a commercial scale as it is labour intensive.

Plants had to be replaced periodically to maintain fast growth and to avoid fouling by epiphytes which would lower the market value. Unlike ***Gracilaria***, ***Chondrus*** and ***Eucheuma*** which continuously have the ability to regenerate vegetatively (Neish and Fox, 1971; Goldstein, 1973; Edelstein, 1977; Doty and Alvarez, 1975), ***Palmaria*** is only able to do that at a certain time. In ***Palmaria*** marginal proliferations only occur during winter and spring (Rosenvinge, 1931; Guiry, 1976). That means that new plants had to be brought in from somewhere to replace those harvested.

In this study the new plants were obtained from the sea. Apart from the cost of collecting new plants, in the long term this may reduce the wild population.

At present the inability of ***Palmaria*** to produce marginal proliferations in the outdoor tank at some times of the year could be the major constraint in mariculture programme.

CHAPTER THREE

Growth pattern of *Palmaria palmata* under laboratory conditions

Introduction

Growth of seaweeds can be expressed in terms of increase in number of cells per plant (Boney and Corner, 1962; Burns and Mathieson, 1972), increase in length (Rueness and Tananger, 1984; Kain, 1987), increase in area (Grandy, 1984; Hansen, 1977) and increase in weight (Penniman *et al.*, 1986; Morgan and Simpson, 1981a,b).

Weight measurement has seemed to be favoured by many workers because of its relative simplicity and because it can be carried out quickly. However, this method is not applicable in every situation especially for *in situ* measurement. In this case another method of measurement must be employed.

Comparison of growth rates between different groups of seaweeds can be very difficult because of different types of measurement employed. Furthermore growth rates are rarely measured in whole plants (Kain, 1987). Different parts of seaweed thalli have different growth rates.

Much of the growth in most red seaweeds, especially in *Palmaria*, seems to be by an apical cell or apical cells (Bold and Wynne, 1978; Guiry, 1976). To what extent does the damage to apical tissues affect the growth of red seaweeds?

To answer the above question and to find out more about the growth of ***Palmaria*** the following topics were investigated:

1) growth rates in term of thickness, length, area and weight in three different parts of the hallus namely tip, middle and base;
2) comparison of growth rate at the centre and at the margin of the thallus;
3) comparison between thalli with damaged tips and normal tips;
4) relationship of growth in terms of length, area and weight.

Materials and Methods

Healthy ***Palmaria*** plants, free from epiphytes, damage and from encrusting bryozoans were obtained from the area described in Chapter Two. Young vegetative ***Palmaria*** plants with simple thalli (Fig. 3.1) about 5 to 12cm in length were used for the study of relative growth rate in terms of thickness, length, area and the comparison of growth rates of plants with damaged tips and normal tips. Slightly older vegetative plants with palmate shape thalli (Fig. 3.2) were used to compare the relative growth rate in terms of length at the margin and at the centre. Whenever possible about 20 to 30 plants were used for each experiment. With the aid of a perspex grid, a series of holes were punched 1cm apart along the thallus using a sawn-off hypodermic needle 1.1mm in diameter resembling a miniature cork borer. The thalli were cleaned and excess water removed with soft tissue paper and weighed. The outline of the area of each plant was traced on to acetate sheet together with the positions of the holes (Fig. 3.3). A fine nylon string was passed through the hole at the base of the thallus with a small plastic tag. The base of the thallus together with the nylon string and plastic tag were wrapped with a small piece of plastic foam and attached to a plastic clip. The purpose was to allow individual plants to be identified, prevent plants from detaching from the clip and minimize pressure damage. The base of the plastic clip was attached to the barrel of 1 cm^3 syringe. Each 1 cm^3 syringe can hold up to four plastic clips. This method has been used successfully in the past on

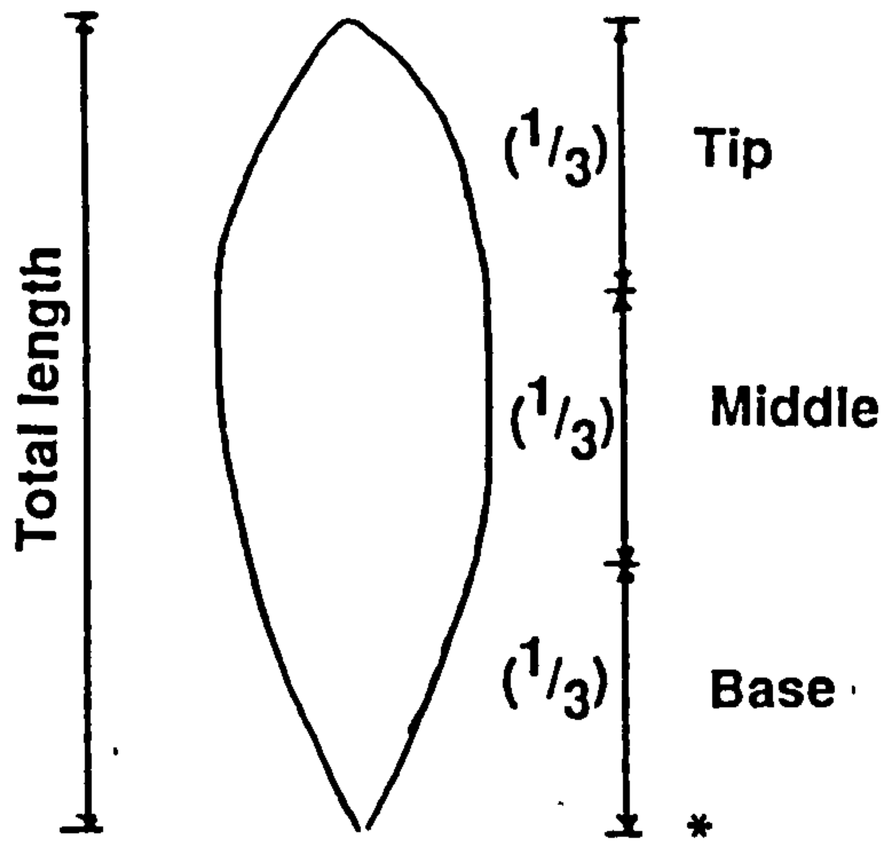


Fig. 3.1. Simple ***Palmaria*** thallus divided into 3 different parts; tip, middle and base for growth pattern study.

* Approximate categories not according to scale.

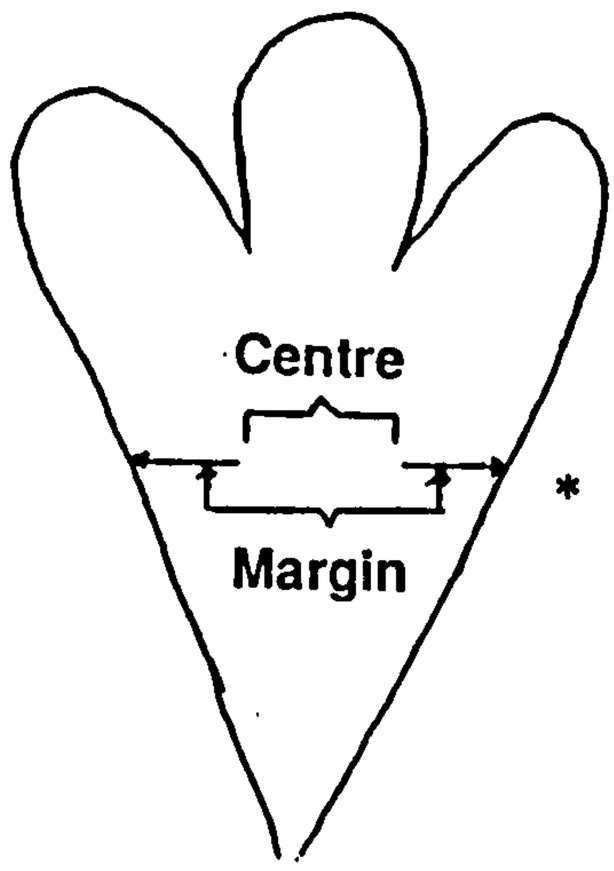


Fig. 3.2. Vegetative ***Palmaria*** thallus with palmate shape showing centre and the margin.

*Approximate categories not according to scale.

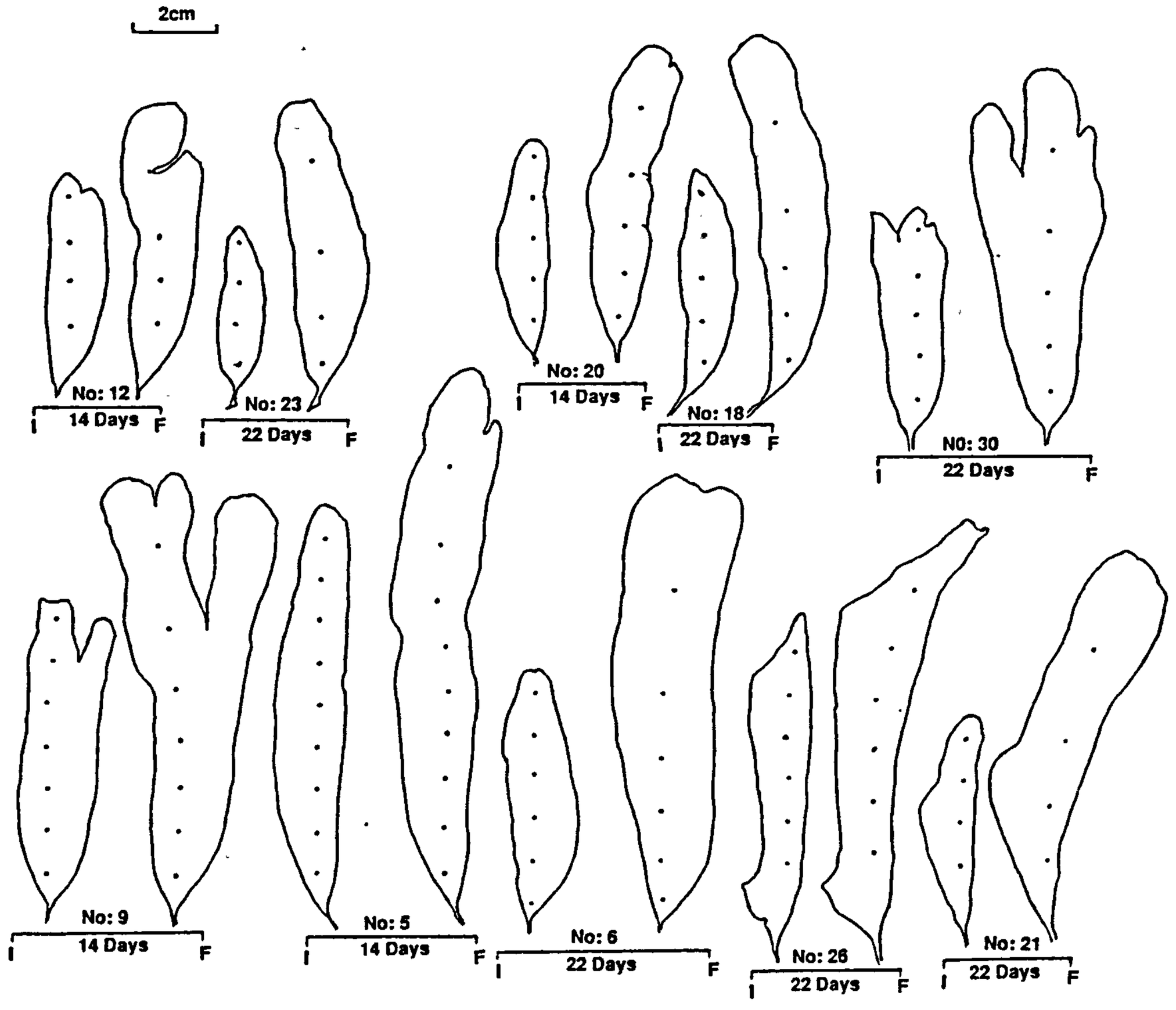


Fig. 3.3. Typical shape of ***Palmaria*** thalli at the beginning and at the end of experiment used for growth studies. I=initial; F=final.

several red seaweeds (Grandy, 1984; Kain, 1987).

Two small submersible pumps were placed diagonally in the tank to create water movement as aeration alone produced localized water movement and pockets of still water. Water was changed every two days to avoid the build up of diatoms, unicellular and filamentous green algae, toxic material, bacteria etc. The tank was pulsed with 0.1mM $NaNO_3$ and 0.01mM K_2HPO_4 (Morgan *et al.*, 1980a) the night before the water was due to change. Light from two 1500mm green Thorn EMI fluorescent 65/80w tubes gave an irradiance of between 45 and 60 $\mu mol\ m^{-2}s^{-1}$ on the surface of the water, the photoperiod was 12:12 h (light:dark) and temperature 12-13^{0}C. Initially each plant was measured every week but later they were measured at the beginning and at the end of the experiment to reduce unnecessary stress to the plant. Experiments lasted from 3 to 5 weeks depending on the conditions of the plants and were carried out during the months of June to September 1986.

From preliminary study, it was found that different parts of ***Palmaria*** blade have different growth rates. Therefore it was decided that the blade was divided into three different regions namely tip, middle and base. The term tip referred to the $^1/_3$ most distal part of the thallus, middle referred to the middle $^1/_3$ of the thallus and base referred to the proximal $^1/_3$, towards the holdfast (Fig. 3.1).

Weight and thickness were measured immediately while area and length measurement were carried out on the acetate sheet tracing itself. The thickness of the thallus was measured using vernier scale Mitutoya No.7321 calipers to the nearest 0.01mm. The area of the tracing was obtained by using a sonic digitizer GRAF/BAR MKII interfaced to BBC Microcomputer Model B. The length of whole thallus and distance between the holes was measured using a Mitutoya dial caliper No. 505.633 to the nearest 0.05mm. The weight was determined to the nearest 0.01g using a Mettler digital balance Model PC3600.

In the field the tips of ***Palmaria*** blades were often damaged or cut off either by grazer or by strong waves action. Therefore to simulate ***Palmaria*** plants with partially and severely damaged tips, apical parts of about 5-10mm and 1/2 of the total length of the blade respectively were cut off (Fig. 3.4a,b).

Calculation for mean relative growth rate per day was based on the equation described in Chapter One and Two with minor adaptation.

$$RT1\text{-}T2 = \frac{LnX2 - LnX1}{T2\text{-}T1}$$

Where R= mean relative growth rate per day (R^A in area, R^W in weight, R^L in length or R^T in thickness)

X1= (area, weight, length or thickness) at time T1

X2= (area, weight, length or thickness) at time T2

"Length" was a linear measurement of the blade in any direction (Kain, 1976). The R^L and R^A for whole plants were calculated from the initial and final total lengths and areas. Computation of results was carried out on IBM mainframe 3083 using MINITAB andSPSSX statistical packages. For multiple comparison between treatments Tukey multiple comparison (Zar, 1984) was employed.

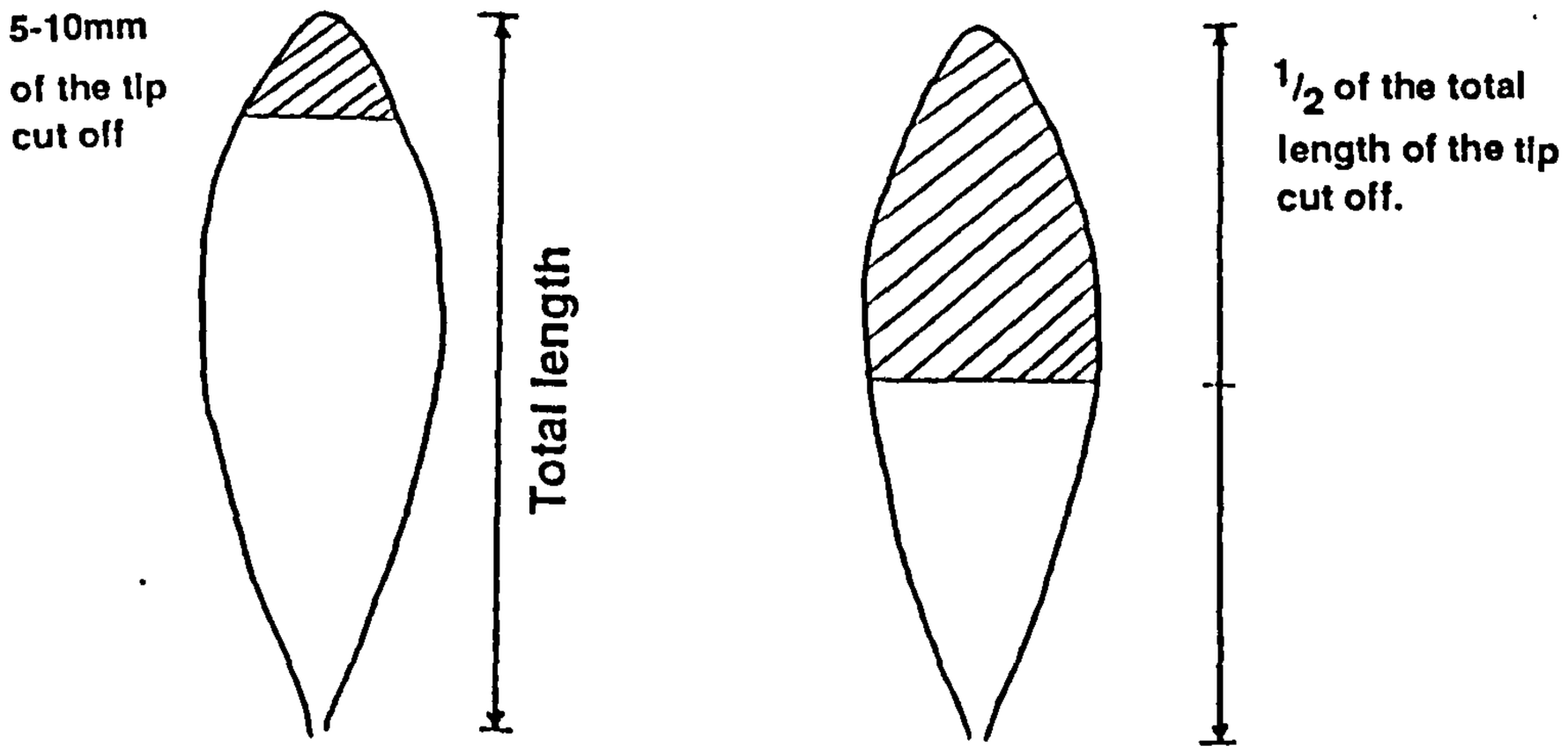


Fig. 3.4. Part of the ***Palmaria*** tip was cut off to simulate

a) partially damaged tip,

b) severely damaged tip.

(Drawing not to scale)

Results

The same sets of ***Palmaria*** plants were used to determine R^T, R^L, R^A and R^W to avoid any variation and enable these growth rates to be compared. Since it is inappropriate to get the R^T of the whole plant, only R^T of 3 different parts were plotted. Similarly for R^W, only R^W of the whole plants were plotted. The R^L, R^A and R^W of the whole plants were plotted against the initial size. Growth in terms of R^L, R^A and R^W decreased significantly as initial size increased (Fig. 3.5, Table 3.1).

Category	Coefficient of determination r^2	ANOVA test P<0.005
R^L vs initial length	0.35	+
R^A vs initial area	0.42	+
R^W vs initial weight	0.37	+

+ significantly different

Table 3.1. Coefficient of determination and ANOVA test showing the significance of correlation between mean relative growth rate (R^L, R^A and R^W) and initial size of ***Palmaria*** plants.

The R^T, R^L and R^A of ***Palmaria*** thalli were highest at the tip followed by the middle while the base had the lowest growth rate (Fig. 3.6 a,b,c). These observations are statistically significant (Table 3.2, 3.3).

At the base the R^T was higher than R^L or R^A (Fig. 3.6a,b,c) in spite of the fact that the thallus is thickest at the base. Although in general the R at the tip and the middle were higher than at the base, there is an exception particularly when the tissues at the tip or at the middle were damaged physically or by bacterial infections as

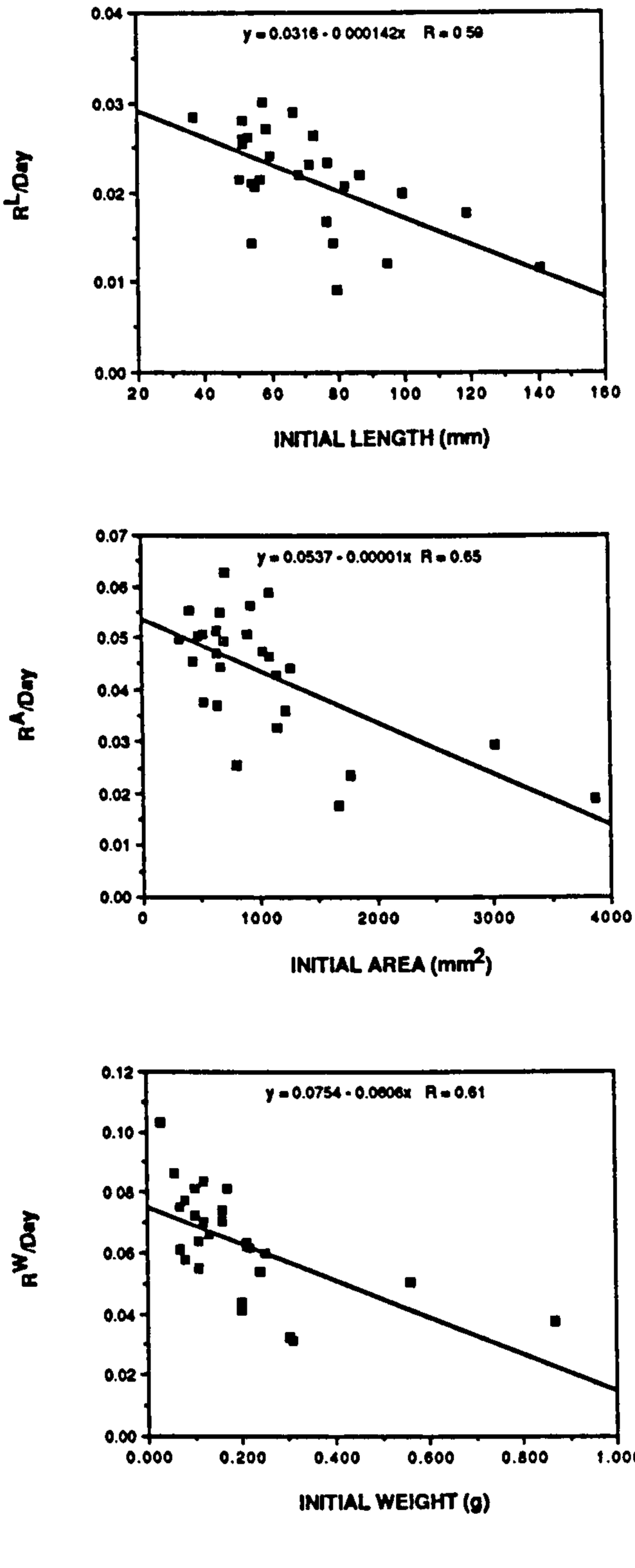


Fig. 3.5. R^L, R^A and R^W versus the initial size of whole ***Palmaria*** thalli in terms of length, area and weight.

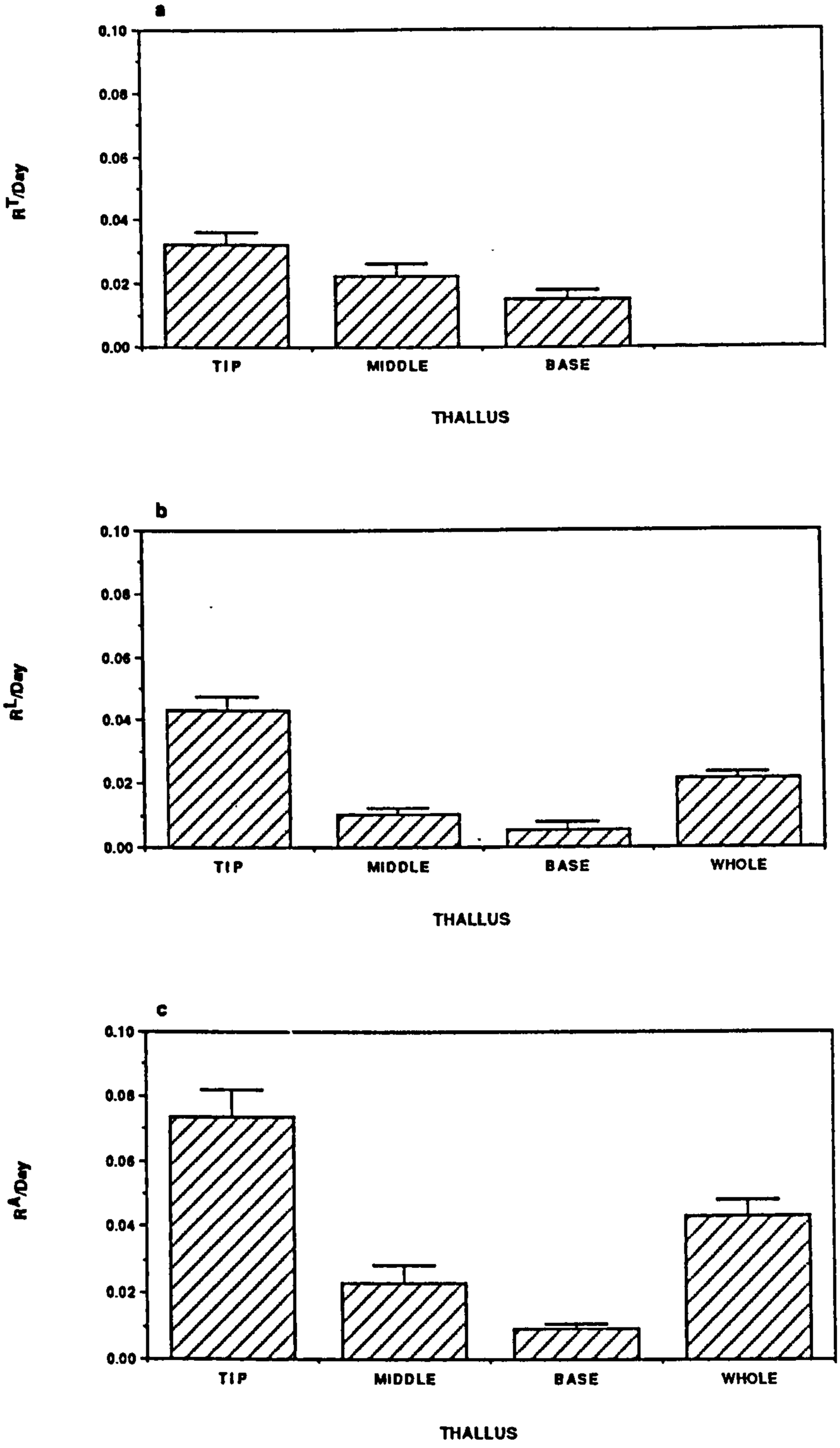


Fig. 3.6. R^{T}, R^{L} and R^{A} from 3 different parts of ***Palmaria*** thalli and R^{L} and R^{A} of the whole thalli with 95% confidence interval.

Category	D.F Group	D.F Error	F	P	Conclusion
R^T	2	84	30.08	P<0.000	+++
R^A	3	116	119.01	P<0.000	+++
R^L	3	116	141.94	P<0.000	+++

+++ Highly significant

Table 3.2. Summary of analysis of variance of R^T, R^L and R^A from three different parts of ***Palmaria*** thalli (tip, middle and base)

Comparison of mean relative growth rate	Conclusion		
	Thickness	Length	Area
Tip vs Base	+	+	+
Tip vs Middle	o	+	+
Middle vs Base	o	o	o

+ Significantly higher than middle or base

o Not significantly different

Table 3.3. Tukey multiple comparison at P<0.05 level of confidence for R^T, R^L and R^A of different part of ***Palmaria*** thalli (tip, middle and base).

shown in Appendix 4 and 5 in which R at the base was higher than R at the middle.

The R^L and R^A at the tip were higher than R^L and R^A for the whole plants (Fig. 3.6 b,c). When the R^L at the tip, the middle and the base were expressed as a ratio of R^L of the whole thallus the growth at the tip was about twice, the middle about half and the base about a quarter the overall growth. The R^A also showed a similar pattern (Table 3.4) (Appendix 4,5).

Measurement	Mean relative growth rate			
	Whole thallus	Tip	Middle	Base
Length R^L	0.022	0.043 (2.00)	0.010 (0.47)	0.005 (0.25)
Area R^A	0.043	0.074 (1.70)	0.023 (0.543)	0.009 (0.21)

Table 3.4. R^L and R^A of different parts of ***Palmaria*** thalli (tip, middle and base) expressed as ratio of R for the whole thalli (in brackets).

Since different parts of ***Palmaria*** thallus had different R^T, R^L, and R^A, it was decided that the R^L of ***Palmaria*** at regular intervals from the tip toward the base should be investigated. However there were several problems in determining the R^L at regular intervals from the tip toward the base. Firstly plants of different sizes were used. It was found that growth of ***Palmaria*** declined as the plants became larger. Since the plants of different sizes were used, the distance and the number of holes punched along the blade from tip toward the base were not equal (Fig. 3.7). Therefore it is necessary to represent R^L between the punched holes from the tip toward the base of the plants of different sizes proportionally. To achieve this the R^L

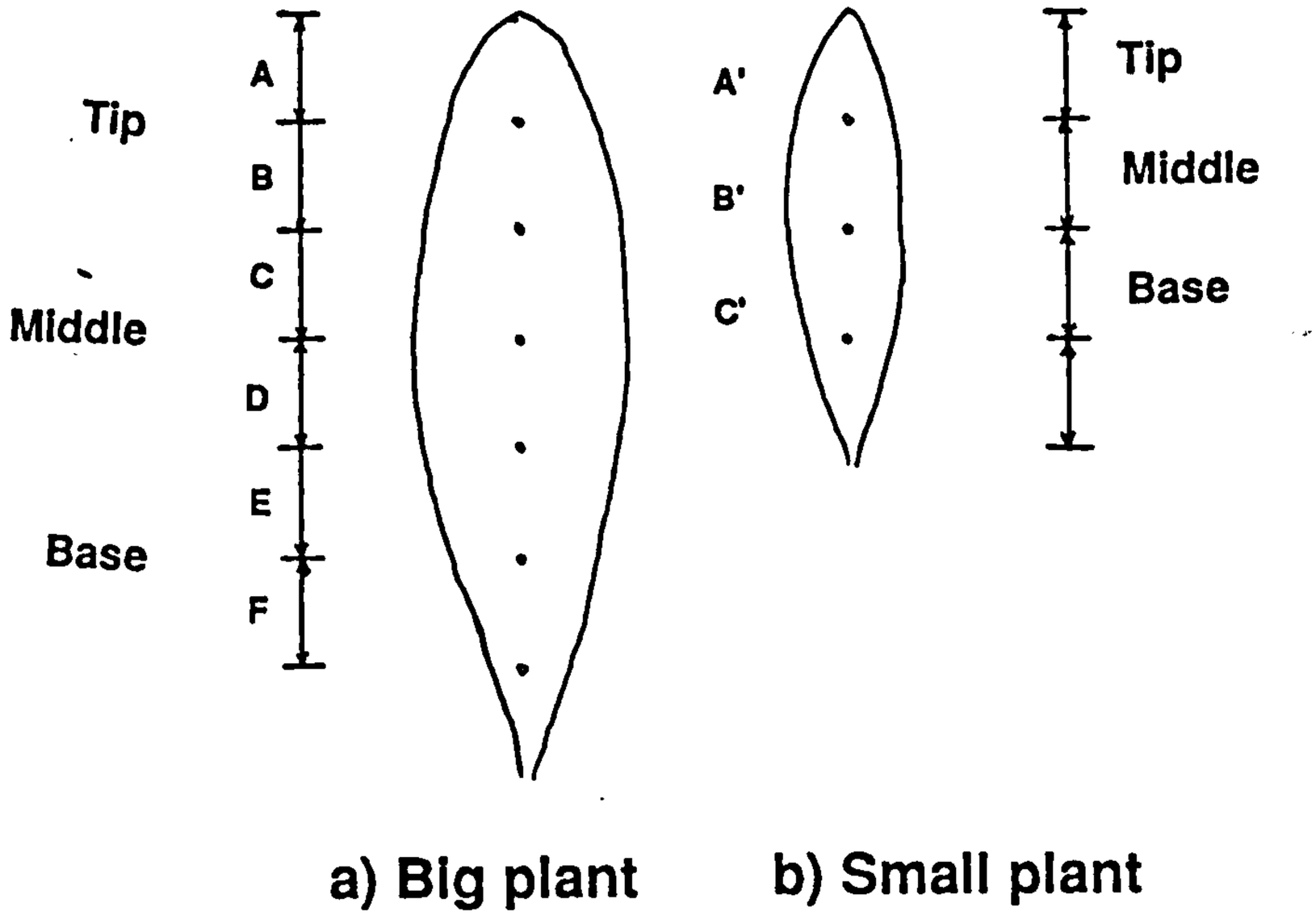


Fig. 3.7. Illustration of *Palmaria* plants of different sizes used for the study of R^L measured between the punched holes from tip to base.

between each point along the thallus was divided by the total length of the individual plant. The R^L from 28 plants were plotted against the log of the proportional distance from the tip to the base (log Tipbase) to get the linear relationship.

The R^L decreased as the distance moved further away from the tip toward the base with regression equation Y=- 0.00271 - 0.06174(log Tipbase) (Fig. 3.8). ANOVA test shows that the relationship (between R^L and log Tipbase is highly significant at $P<0.000$ with coefficient of determination $r^2= 0.82$.

In young ***Palmaria*** plants with a simple thallus, the relationship of R^L for the length ($R^L L$) and R^L for the width ($R^L W$) of the blade was investigated to find whether the linear growth rate is faster than the width growth rate. $R^L L$ was determined by the distance between the punched holes (Fig. 3.9). Determination of $R^L W$ was more complicated because of the changing shape of the thallus as it grows. There is a need to find a reliable reference point in which the width of the thallus can be measured. Measuring the width of the thallus by drawing a line perpendicular to the imaginary vertical axis is not reliable because it did not offer fixed reference point and changing of the imaginary vertical axis as the thallus grow and change shape. One of the best ways to determine $R^L W$ was to draw a line across the hole perpendicular to one side of the margin because it offer fixed reference point (Fig. 3.9). With the exception of $R^L W$ near the tip and the base, $R^L L$ was plotted against the mean of two $R^L W$.

Since the growth rates at the tip, the middle and the base were different, the $R^L L$ and $R^L W$ at the tip, middle and base were separated and plotted (Fig. 3.10). At the tip $R^L L$ grew faster than $R^L W$ with regression coefficient Y= -0.00490 + 1.74($R^L W$) and coefficient of determination $r^2= 0.61$. At the middle $R^L W$ grew faster than $R^L L$ with regression coefficient Y= 0.00214 + 0.620($R^L W$) and coefficient of determination $r^2= 0.61$. At the base both $R^L L$ and $R^L W$ were very low and there was no obvious relationship between $R^L L$ and $R^L W$.

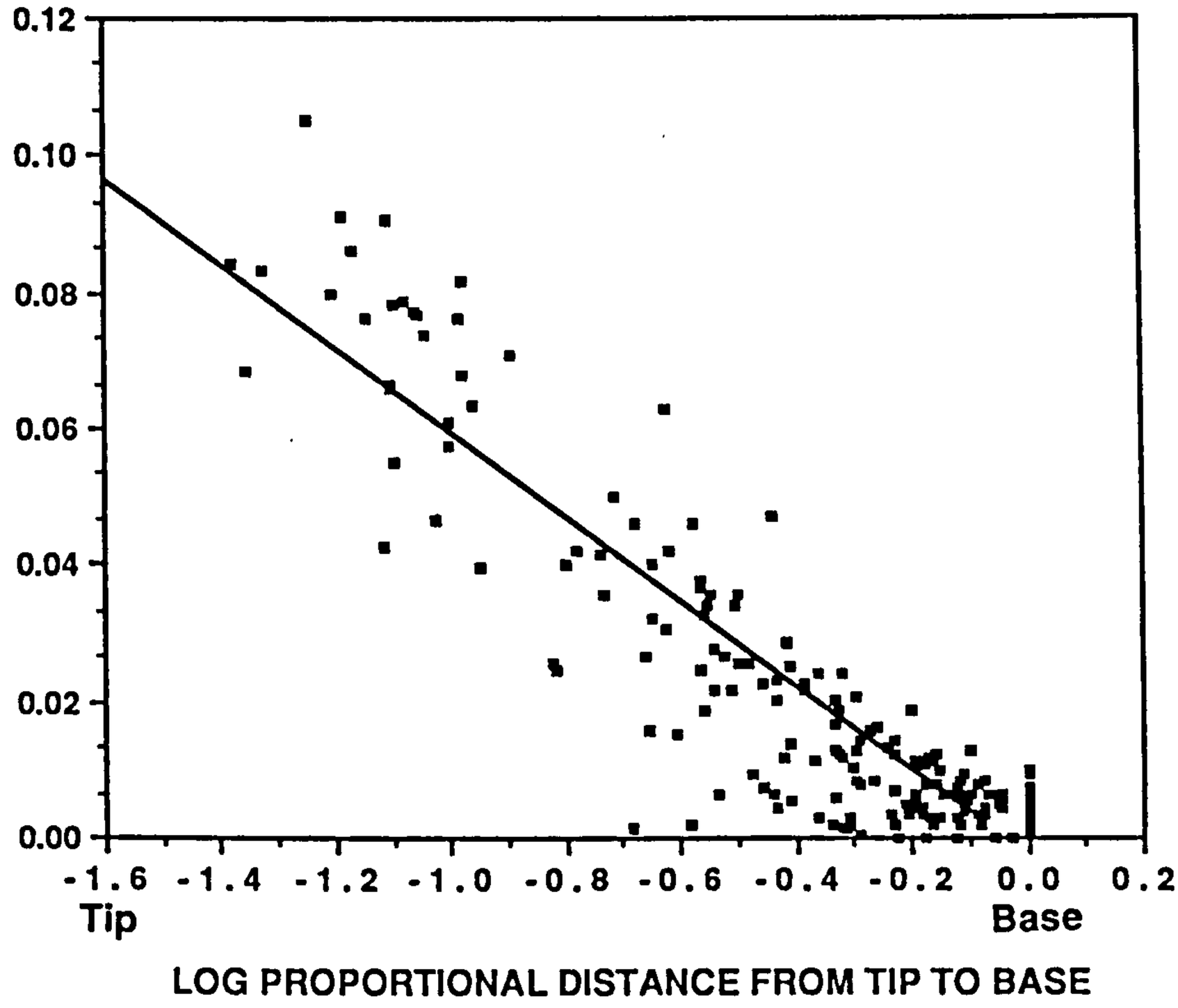
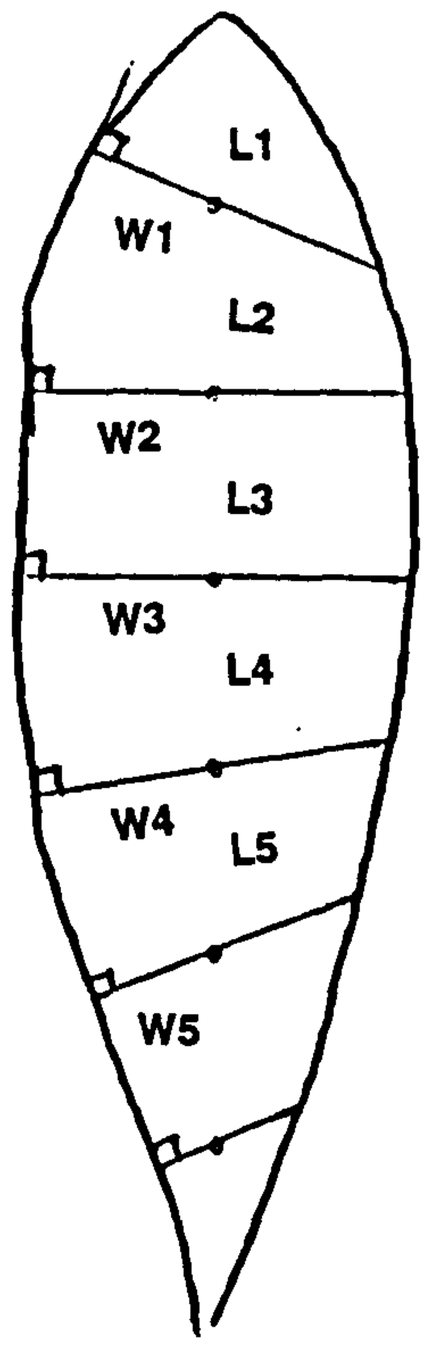


Fig. 3.8. Log R^L versus log of the proportional distance from tip to base of *Palmaria* thalli.

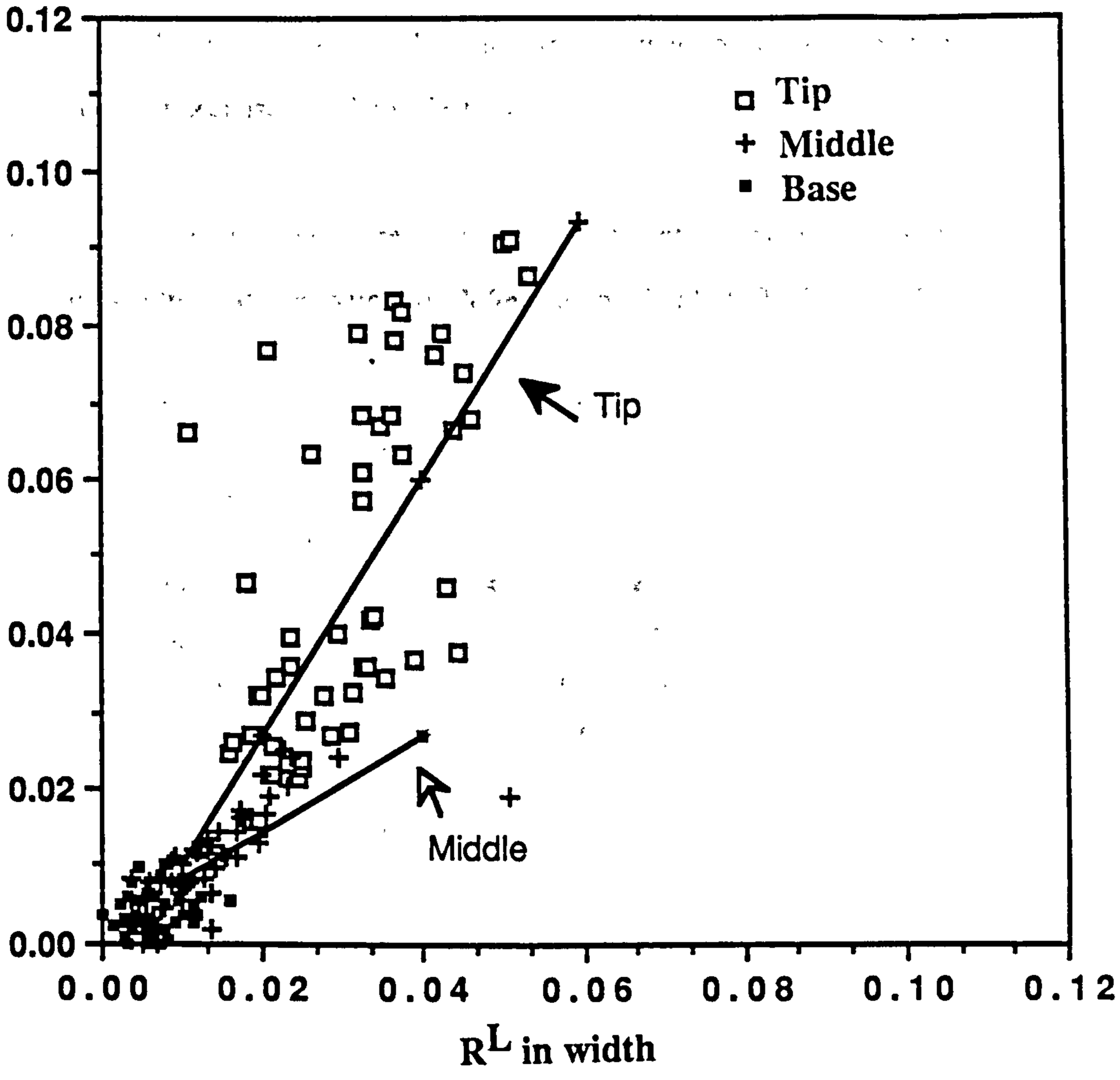


$(R^{L}L1)$ AND $(R^{L}W1)$

$(R^{L}L2)$ AND $(R^{L}\frac{W1+W2}{2})$

$(R^{L}L3)$ AND $(R^{L}\frac{W2+W3}{2})$

Fig. 3.9. Illustration of how $R^{L}L$ and $R^{L}W$ of ***Palmaria*** thallus was measured.



Tip $y = -0.005 + 1.71x$ $R = 0.78$

Middle $y = 0.002 + 0.620x$ $R = 0.78$

Fig. 3.10. Relationship between R^L in length and R^L in width at the tip, middle and base of simple ***Palmaria*** thallus.

In ***Palmaria*** plants with a palmate thallus, a comparison was made to find out whether the R^L at the centre of the thallus was faster than at the margin. Mean R^L at the centre was compared with mean R^L at the margin (Fig. 3.2). Most of the comparisons of R^L at the centre and at the margin were only possible at the middle parts of the plant because it is the widest.

Student's t-test showed that there was no significant difference in growth rates between the centre and the margin of ***Palmaria*** thalli at $P<0.05$ (Table 3.5).

RL	Margin	Centre
Sample size	30	30
Mean	0.00885	0.00839
S.D	0.00701	0.00539
t-statistic	0.28	Hypothesis HO:U1=U2 HA:U1=U2
Conclusion: accept HO at $P<0.05$		

Table 3.5. Student's t-test of R^L at the centre and the margin of ***Palmaria*** thalli.

The R of young ***Palmaria*** thalli with partially damaged tips and normal tipped thalli were compared to see how this affected growth in term of length, area and weight (Fig. 3.11). Tracings of ***Palmaria*** plants showed that plants managed to grow behind the cut tips (Fig. 3.12). Student's t-test showed that there is no significant difference in R^A and R^W between partially damaged tip and normal tip but the R^L was significantly different (Table 3.6).

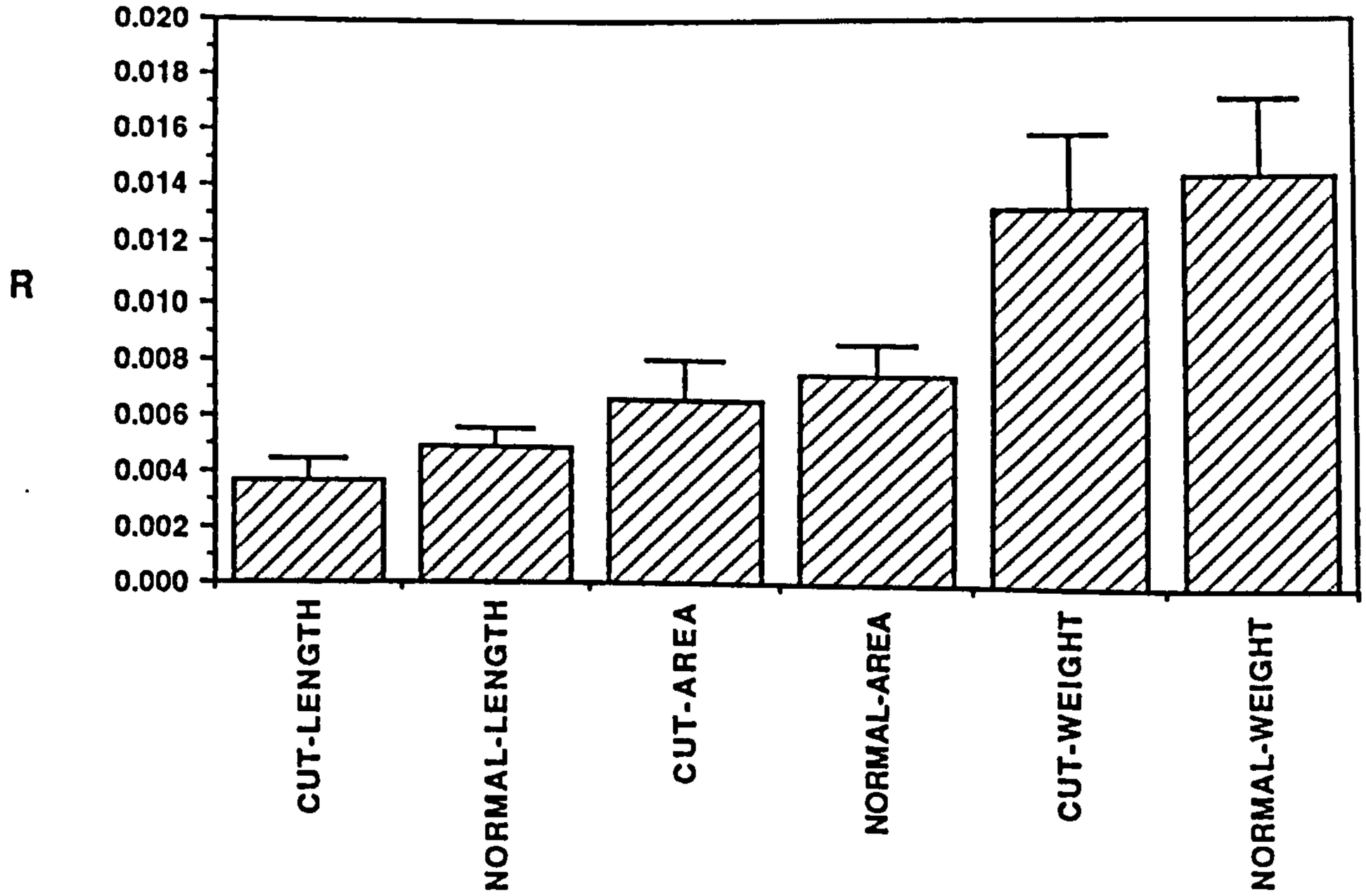
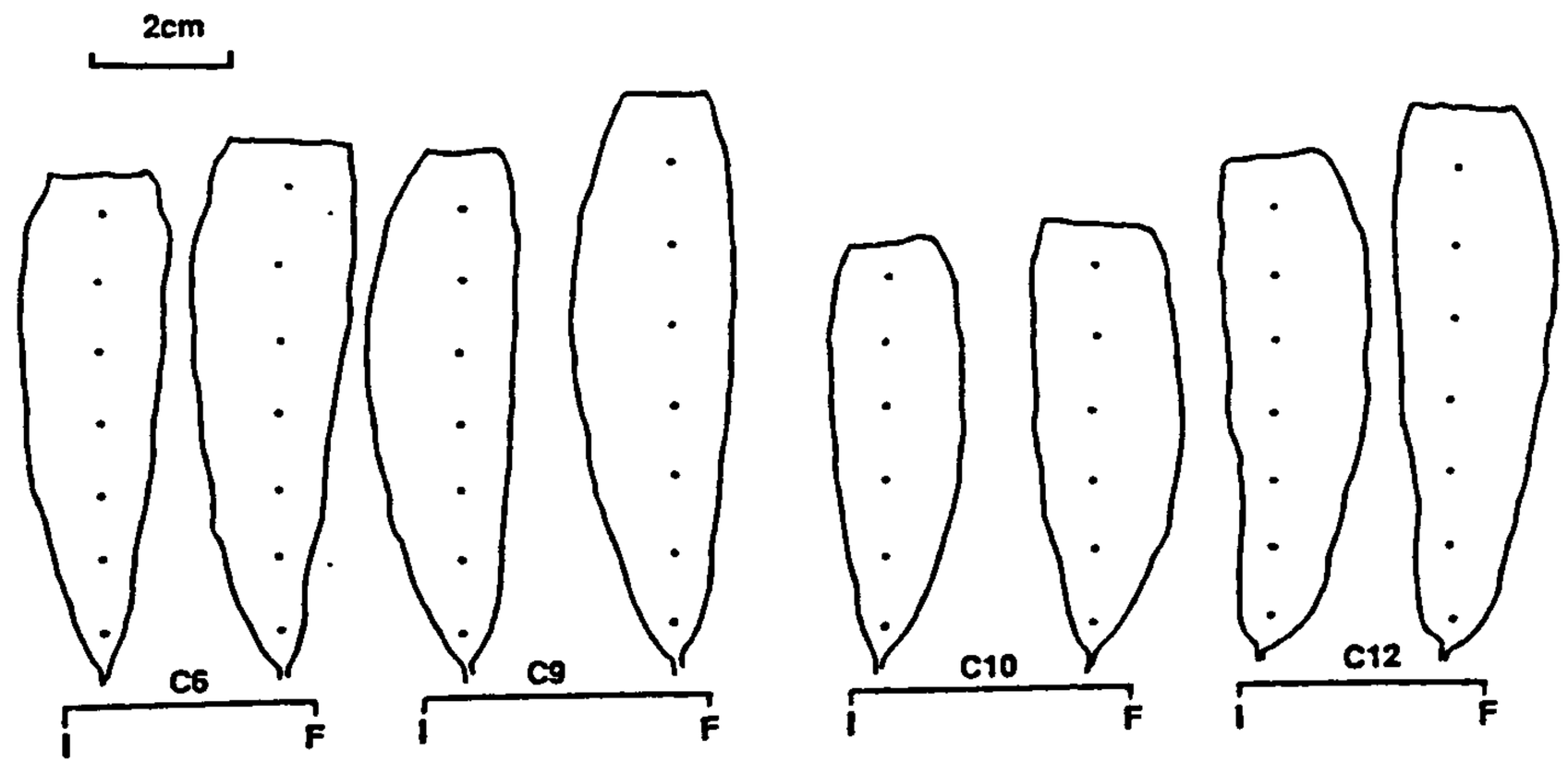
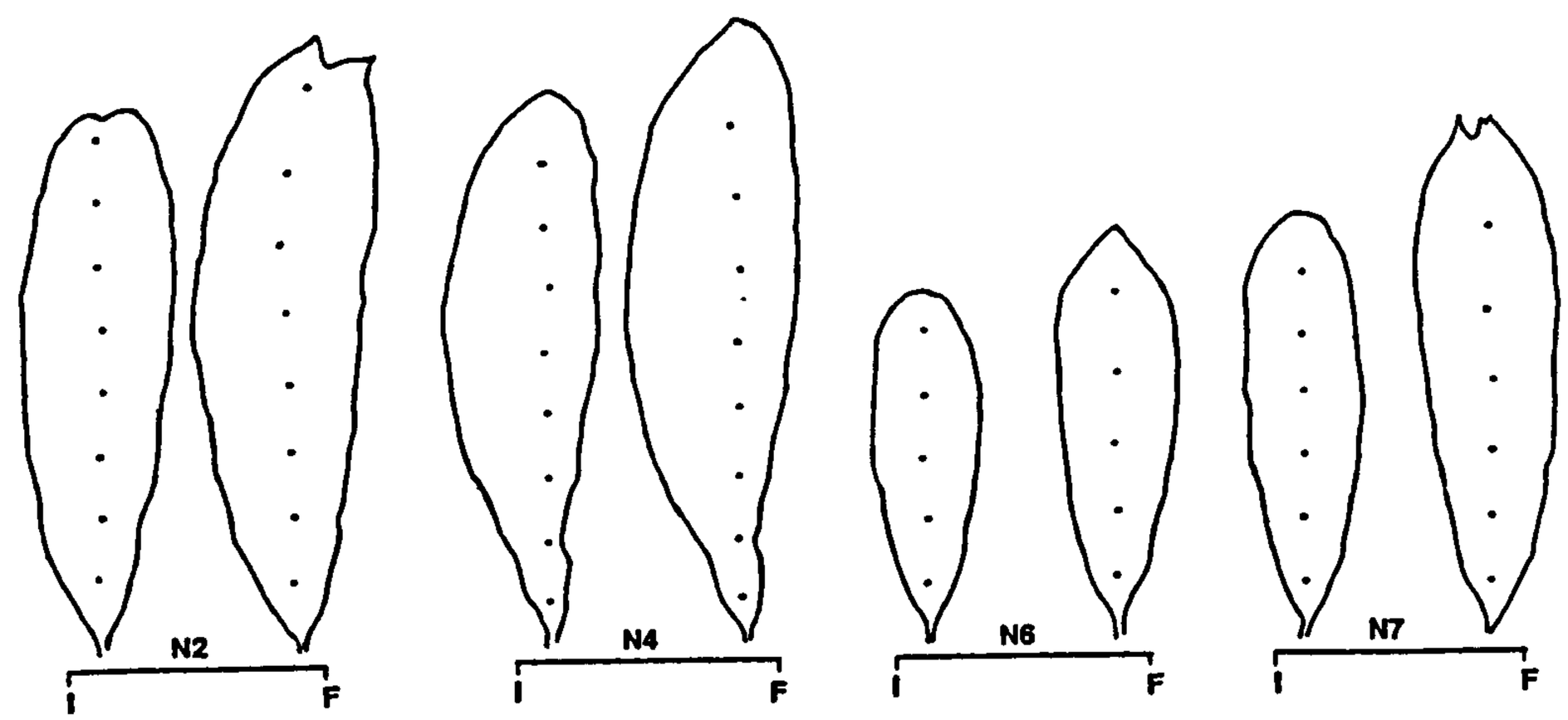


Fig. 3.11. Comparison of R^L, R^A and R^W of ***Palmaria*** thalli with partially damaged tips and normal tips with 95% confidence interval.



B



A

Fig. 3.12. Tracing of ***Palmaria*** thalli with A) normal and B) partially damage tips at the beginning and at the end of the experiment (22 days). I=initial; F=final

Variable (mean in bracket)	D.F	t-test	Conclusion
Area Cut tip vs Normal tip (0.00663) (0.00758)	52	1.07	*
Length Cut tip vs Normal tip (0.00365) (0.00483)	52	2.40	+
Weight Cut tip vs Normal Tip (0.01353) (0.01463)	52	0.62	*

(*) not significant

(+) significantly lower at $P<0.05$

Table 3.6. student's t-test of R^L, R^A and R^W of partially damaged tip and normal tip of ***Palmaria*** thalli.

Although the R^L with partially damaged tips were significantly lower than with normal tips, the plants still showed positive growth rates (Fig. 3.11, Appendix 6).

Since the partially damaged tips did not significantly slow down R^A and R^W, a scaled down experiment was carried out to find out if culturing thalli with the apical half removed would have any effect on R^W. Group one with half of the total length of the apical segment cut off to simulate severely damaged tips and group two with normal tips (Fig. 3.4b). They were grown in the same tank to ensure identical culture conditions. Because in the first experimen' R^W was less affected by partially damaged tips (Table 3.6), it was decided that only R^W would be measured and compared.

Student's t-test showed that R^W of severely damaged tip was significantly lower than the normal tip with $P< 0.001$ (Fig. 3.13, Table 3.7).

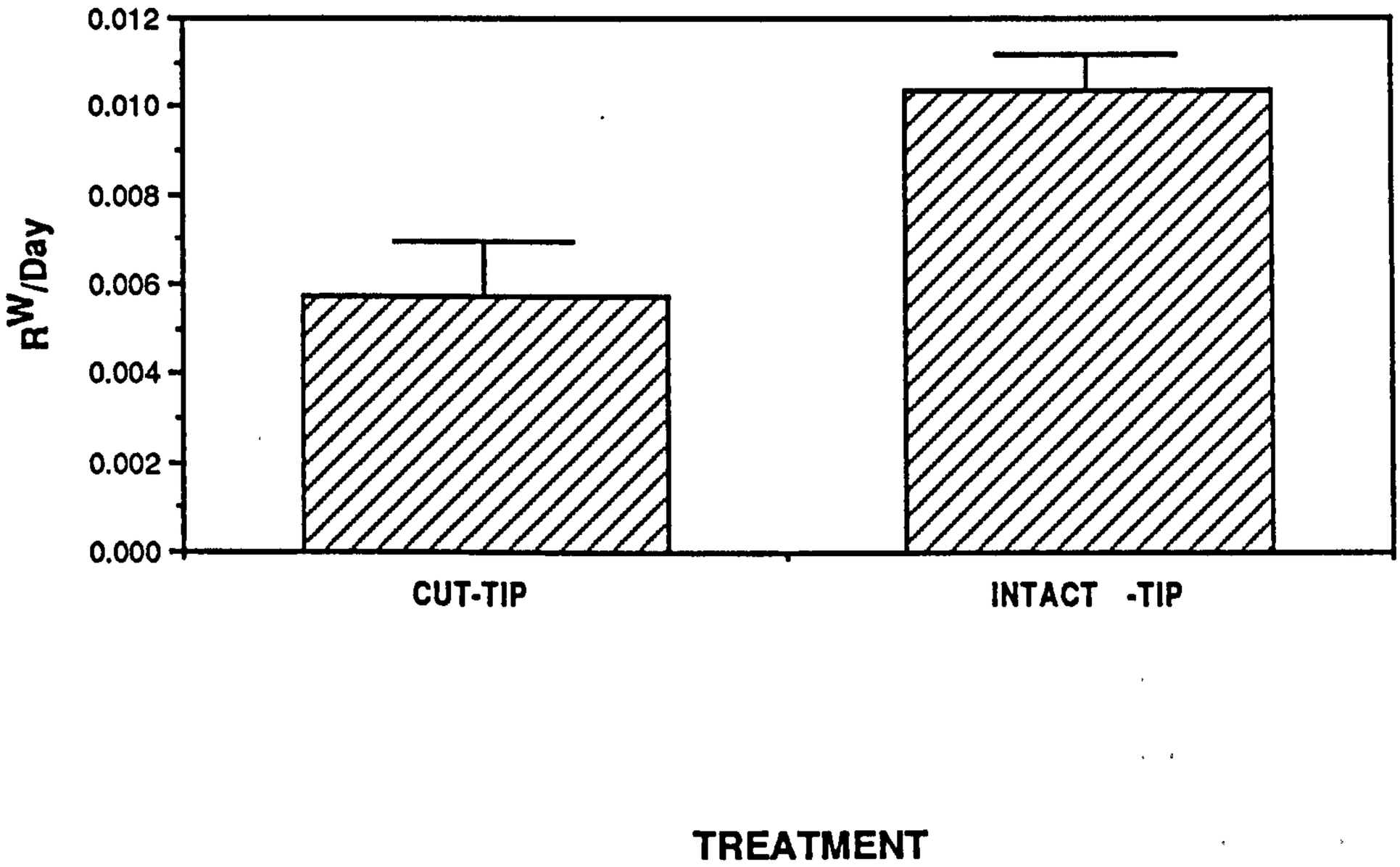


Fig. 3.13. Comparison of R^W of ***Palmaria*** plants with normal tips and severely damaged tips with 95% confidence interval.

R^W damaged tips	Severely	Normal tips
Number of plants	30	30
Mean	0.00575	0.01039
S.D	0.00212	0.00315
t-statistic	6.70	Hypothesis HO:U1=U2 HA:U1=U2

Conclusion: reject HO at P<0.001

Table 3.7. Comparison of R^W of severely damaged and normal tips of ***Palmaria*** thalli using Student's t-test.

The R^L, R^A and R^W of whole simple ***Palmaria*** thalli were plotted and their means were calculated (Fig. 3.14). The means of R^L, R^A and R^W for the whole thallus were 0.02152, 0.04323 and 0.06364 respectively. To find the relationship between R^W, R^A and R^L, R^W and R^A were plotted against R^L (Fig. 3.15). R^W and R^A were respectively 2.79 and 1.99 times that of R^L.

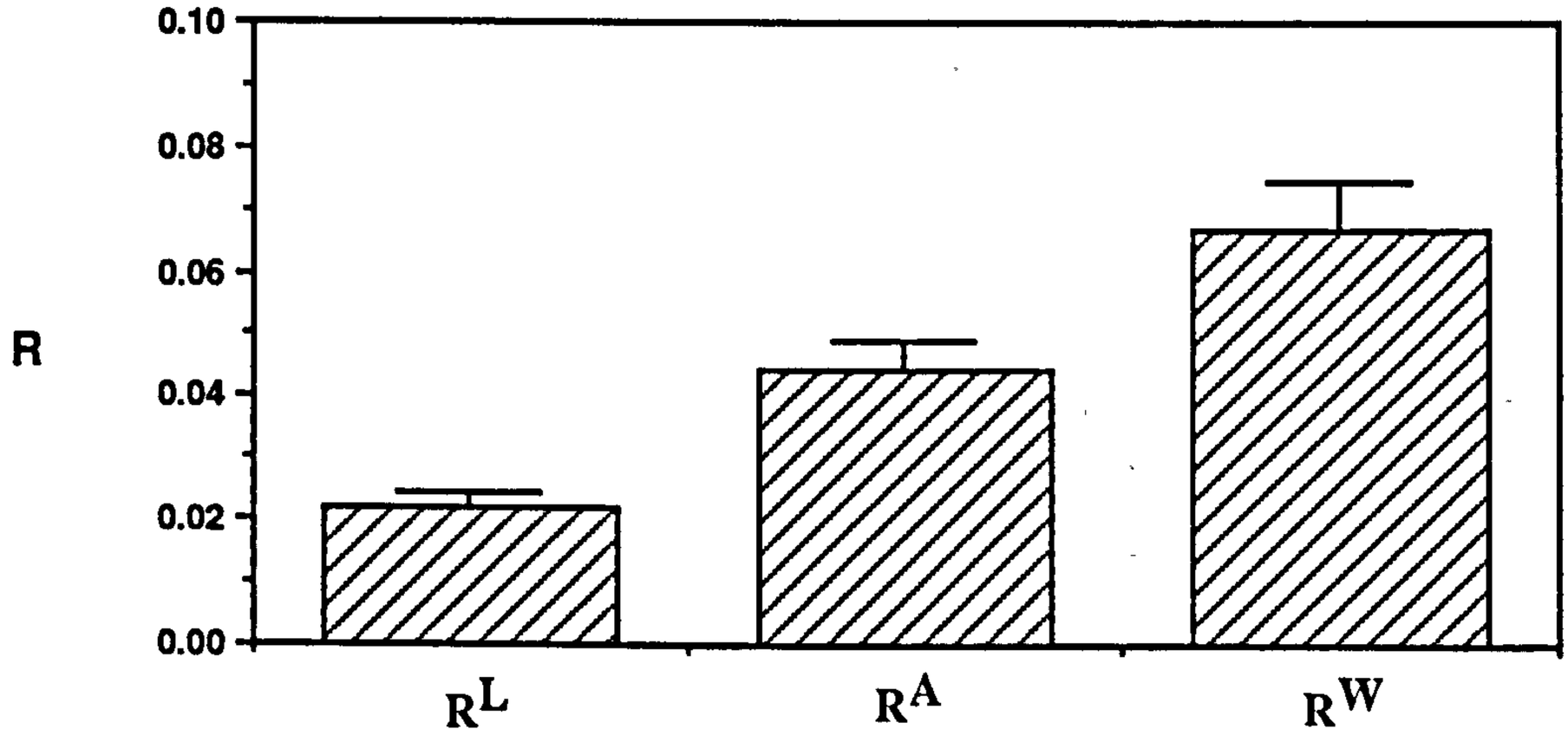
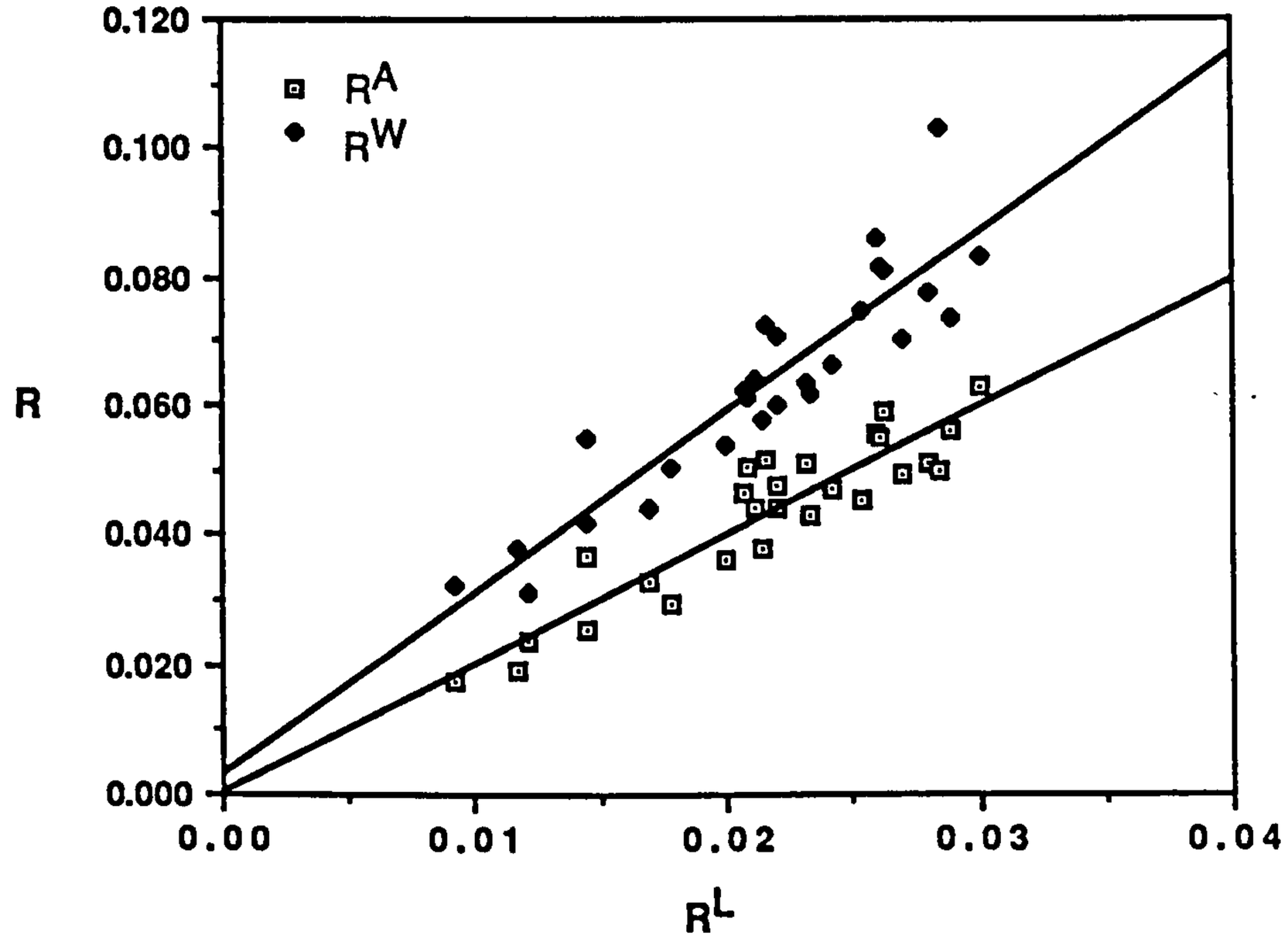


Fig. 3.14. Comparison of R^L, R^A and R^W of whole ***Palmaria*** thalli with 95% confidence interval.



R^A: y=0.00029 + 1.99x R=0.92

R^W: y=0.0034 + 2.79x R=0.91

Fig. 3.15. R^W and R^A versus R^L of the whole *Palmaria* thalli.

Discussion

It has been mentioned earlier that growth of most red seaweeds, ***Palmaria*** in particular, seemed to be by an apical cell or apical cells. Growth in terms of thickness, length and area were fastest at the tip and gradually slowed towards the base. The R^L measured at 1cm intervals from the tip towards the base showed that growth decreased as it moved away from the tip.

Although growth activity in terms of thickness, length and area seemed to concentrate near the tip there was less relative drop in R^T than R^L or R^A towards the base (Fig. 3.6 a,b,c). Thus while the apical part is expanding laterally the basal part is mainly becoming thicker.

The thallus is composed of a cortex of 2-3 layers of cell when young increasing to 20 or more layers when older. In the older part of the thallus, moving away from the tip, the surface cortical cells divide repeatedly periclinally, forming rows of similarly-sized cells which are anticlinally oriented, accounting for increase in thickness toward the base of the thallus (Guiry, 1976). Under normal growing conditions when the ***Palmaria*** thallus is not damaged, the relative growth rate in area and length in the middle and at the tip is greater than the base. This is probably part of ithe growing strategy which gives priority for the middle and the tip to increase surface area to optimize nutrient uptake and photosynthesis. Increase in area at the middle and at the tip will also increase the drag force caused by water movement and a thicker thallus at the base will reduce the chances the thallus of being damaged or breaking off from the holdfast.

In young ***Palmaria*** plants grown in laboratory, $R^L L$ grew faster than $R^L W$ at the tips giving the plants tapered tips. At the middle $R^L W$ grew faster than $R^L L$ which gave plants a broader appearance. No comparison was made between $R^L L$ and $R^L W$ at the

base because of very little growth. The overall shape of young a ***Palmaria*** plant is tapered at both end and broad in the middle which supports the observation made by Guiry (1976) who described young ***Palmaria*** as an elongated-oval blade. In older plants the direction of growth depends on the environmental conditions where they live. In sheltered areas, the width will probably grow more than the length in most parts of the blade which gives the plant a more palmate shape whereas in plants grown in fast moving water, the length will probably grow faster than the width giving an elongated streamlined blade (Rosenvinge, 1931).

It is a common occurrence that ***Palmaria*** plants with damaged apices continue to grow and increase in size (Rosenvinge, 1931; Guiry, 1976; Grandy, 1984) and often produce marginal proliferations in the second year of growth.

Mature ***Palmaria*** thalli posses a marginal meristem (Fritsch, 1945). The fact that ***Palmaria*** with damaged tips still shows lateral growth gives a strong indication that some meristematic tissue must be located behind the tip. It would appear therefore, that providing the meristematic tissues are not completely destroyed, the plant still has the ability to grow (Grandy, 1984). Guiry (1976) observed growth and differentiation typically taking place over a broad area at the apices; when apical dominance is broken several to many subapical meristematic regions are developed which are typically at the truncated apices, at the margins of the fronds and on the basal disc. Under certain ecological conditions numerous localised meristematic regions along the margins of the thalli are developed and, depending on the rate of growth and other factors, give many different blade shapes. Such thalli may have irregularly serrate margins as a result.

It has been mentioned earlier that partial damage to tips of ***Palmaria*** affects the R^L significantly more than the R^A and R^W. This is because the plant can increase in area behind the partially damage apices (Fig. 3.15).

The relationship between R^L, R^A and R^W can be explained mathematically. Theoretically if the shape remained unchanged, two-fold increase in length will increase the area by four-fold and increase the volume by eight-fold (Table 3.8).

Dimension	Initial	After	R theoretical	Ratio to R^L R' observed
Length	1	2	0.693	1.00 1.00
Area	1	4	1.386	2.00 2.01
Volume/Weight	1	8	2.079	3.00 2.96

Table 3.8. Theoretical R^L, R^A and R^W of an imaginary cube shape cell after one time unit. R'= value obtained from this study.

Imagine the theoretical cube-shaped cell mentioned in Table 3.8 is filled with cells and cells have mass. Since weight is equal to mass multiplied by gravity, we might say the volume relates closely to weight. Therefore the ratio for R^L: R^A: R^W is 1: 2: 3 respectively. In a seaweed such as ***Palmaria*** the shape and the thickness of the thallus will change as it grows, it is almost impossible to achieve the theoretical ratio mentioned previously. Results obtained by mean ratio of R^A and R^W to R^L were higher (Table 3.8) than obtained by regression (Fig. 3.15). This is because results obtained by regression take into account of variation of individual R^A and R^W whereas ratio methods only measure the overall effect of R^A and R^W against R^L.

Results from these experiments showed that R^A was about the same but R^W was less than the theoretical ratio (Table 3.8). The results suggest that perhaps growth in terms of area was accompanied by less growth in thickness thus lower R^W than the

theoretical ratio.

Although the R^W was less than the theoretical ratio, growth in terms of weight was faster than area and length one can assume that the plants also increased in thickness as they grew. This study was conducted under laboratory conditions in batch culture, where culture conditions can be stressful to ***Palmaria*** plants (Jones and Dent, 1970): one would expect the growth pattern in the field to vary slightly depending on the environmental conditions which affect its morphology as well as growth rates.

CHAPTER FOUR

Field cultivation of *Palmaria palmata* from spores and from fragments of thallus

Introduction

Over the years various seaweed cultivation techniques have been developed, such as onshore tank culture (Neish *et al.*, 1977; Ryther *et al.*, 1978; Morgan *et al.*, 1980a and Davis, 1980), rope and net culture (Tseng, 1981a; Holt, 1984; Dawes, 1987) and spray culture (Moeller *et al.*, 1984; Lignell and Pedersén, 1986). However not all the developed culture techniques will suit every seaweed as each species has distinct characteristics and biological requirements.

The choice of cultivation methods are partly determined by factors such as the hardiness of the species, availability of sporing materials, spore viability and ability to attached on to artificial substrata, ability to propagate vegetatively and environmental conditions.

Non biological factors such as practicality and production costs which include manpower will eventually dictate the choice of methods for commercial production. Culture methods successful in one area are not necessarily suitable in another area. Cultivation of ***Eucheuma*** for example is very successful in the Philippines (Doty and Alvarez, 1973, 1975) and in some other regions where labour is inexpensive, but is impractical in the developed nations where manpower is expensive, unless the need for intensive labour can be replaced through mechanization or other forms of cultivation methods.

Cultivation of ***Palmaria*** in onshore tanks in the Iasle of Man has been discussed in

Chapter Two. Due to the high cost of maintaining onshore culture tanks and the inability of *Palmaria* to propagate vegetatively, an alternative method of cultivation must be sought.

In situ culture of *Palmaria* from spores by Dion and Delépine (1981) in the field opened the possibility of growing *Palmaria* from spores. The new-found methods developed for keeping sporing materials and obtaining spores (Chapter One) facilitated seeding of spores on to artificial substrata and transplanting them to the sea. Since Dion and Delépine's method was not suitable for large scale *Palmaria* cultivation, the established rope culture method for *Laminaria* in the Irish Sea was adopted (Dawes, 1987). Such a rope system has been shown to be very productive in the east (Tseng, 1981b; Hasegawa, 1971, 1976). *Palmaria* spores were seeded on to different type of cords, incubated in the laboratory and transplanted to the rope system similar to that used by Kain and Dawes (1987).

Marginal proliferations in *Palmaria* occur seasonally and has been observed in the field during late winter and early spring (Rosenvinge, 1931; Guiry, 1976). The possibility of inserting fragments of *Palmaria* thallus into the cords was also investigated. While no holdfast would be formed hopefully the formation of secondary thalli from the older tissues would secure the plant in place. This method is very laborious but if it succeeded, perhaps a mechanical device might be employed in the future.

Materials and methods

4.1. Location and Structure of rope system

Field work was carried out at Bay Fine, 1km south of Port Erin on the Isle of Man 54^0 04.6'N, 4^0 46.2'W. The site was chosen for several reasons. Firstly it was the site of

the previous work on cultivation of *Laminaria* on ropes (Holt, 1984; Dawes, 1987). Knowledge of the area and the presence of anchoring blocks meant that rope systems could be set up easily. Secondly, frequent inspection and maintenance work could be carried out whenever necessary as the site is relatively near to the research station.

The bay has an average depth of 12 to 15m at lowest astronomical tide (LAT) with a maximum tidal range of 5.95m. It is exposed to the wind from the southwest through to the north. Currents of up to about 2.9 ms^{-1} are common at spring tides and run parallel to the coastline except in the southern most corner of the bay where there is frequently an eddy (Fig. 4.1) (Holt, 1984).

The vertical cord system which proved reliable in the previous work (Dawes, 1987) was chosen for the study (Fig. 4.2). It allowed the effects of depth on the growth of *Palmaria* to be studied. The anchoring and horizontal ropes consisted of 14mm spun polypropylene rope. Each anchoring rope was shackled to a tyre filled with concrete weighing between 100 and 250kg underwater. The rope was 35m long on each side of the anchoring point and a 5 litre rigid buoy was tied 10m from the surface to stabilize the tension. The horizontal rope was 60m long with a 27 litre bouy at each end and 10 litre rigid bouys were tied at 4m intervals. The main rope system was towed apart perpendicular to the coastline to face the oncoming waves and to provide the right tension to the system. Loops were placed 1m apart on the horizontal rope to facilitate tying of vertical cords to the horizontal rope and preventing the vertical cords from slipping. The vertical cords were 8m long with 1m allowance for tying to a 1kg concrete weight and to the horizontal rope. Therefore the potential growing part of each vertical cord was 7m.

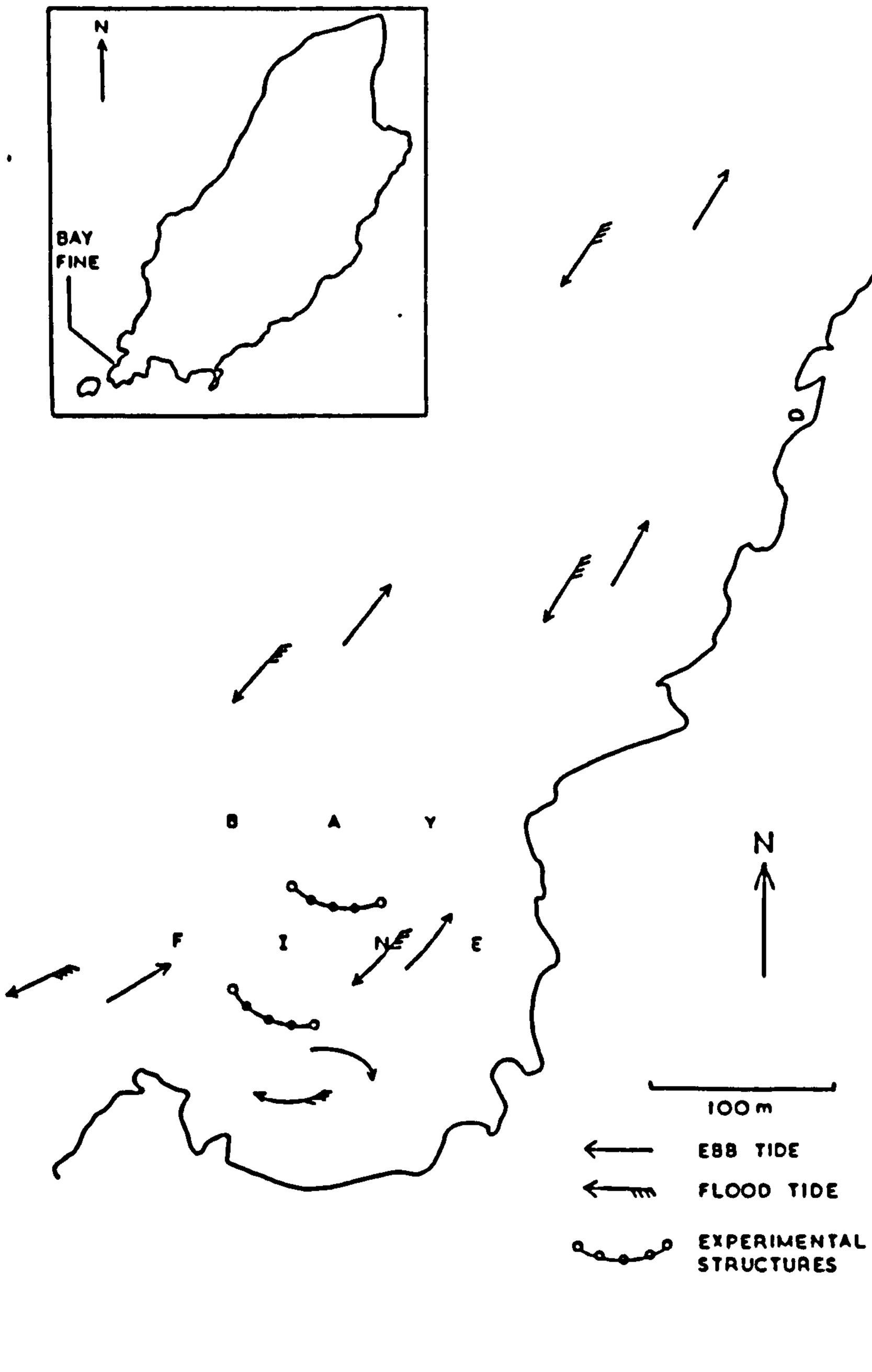


Fig. 4.1. A map of Bay Fine showing tidal flows and typical experimental structures. Inset - a map of the Isle of Man showing the position of Bay Fine (Holt, 1984).

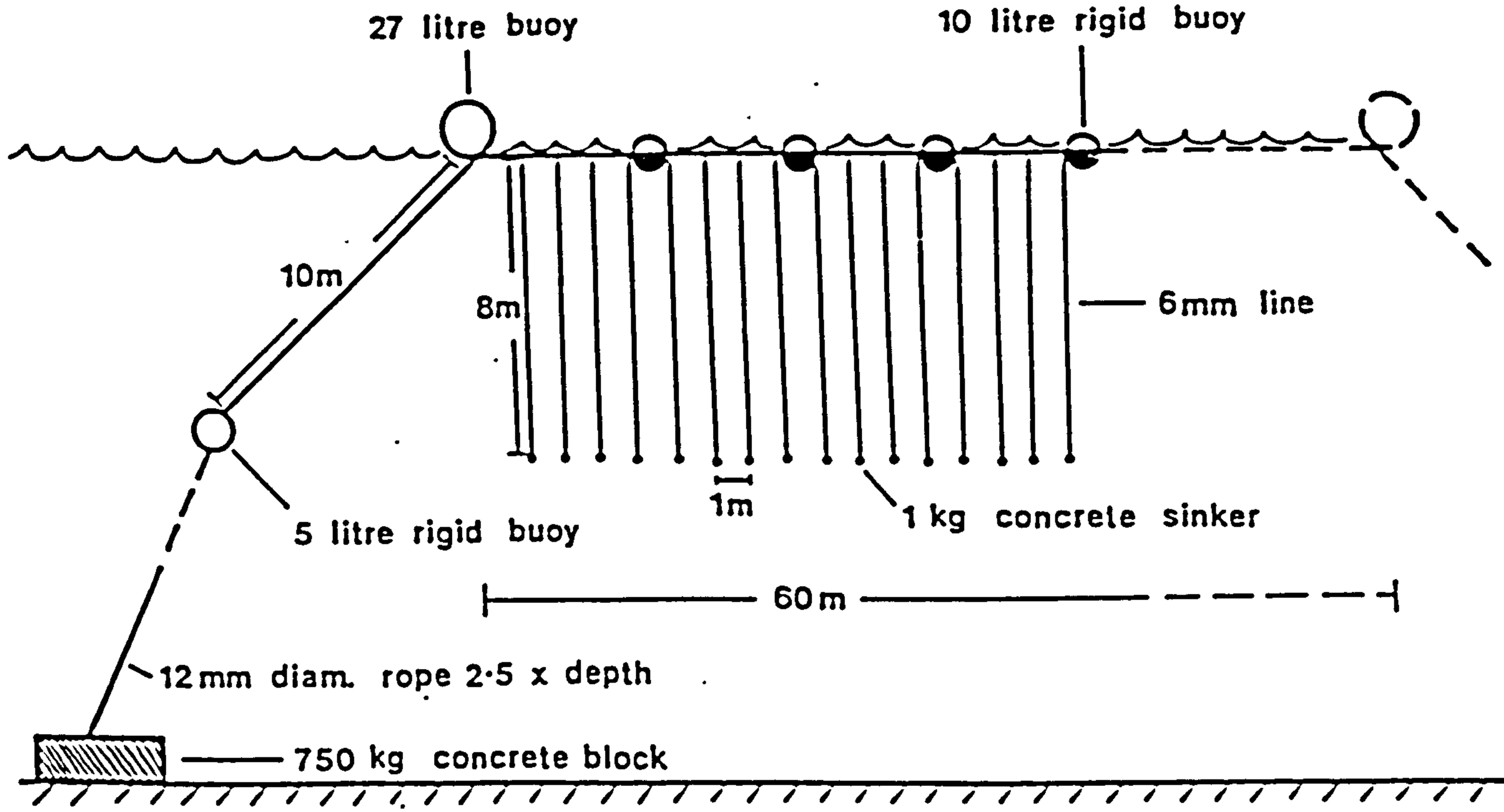


Fig. 4.2. A Diagram of the vertical cord system (not to scale) (Dawes, 1987).

4.2. Seeding of *Palmaria* spores on to the cords

The choice of cords for seeding experiments and for growing fragments of *Palmaria* was based on the rope culture of *Laminaria* in the Isle of Man (Holt 1984; Dawes, 1987). Four different types of cords were tried in this study: 6mm polyethylene cords with smooth twine, 6mm polypropylene with fibrous twine, 3mm braided polyethylene cord and 1.5mm synthetic fibre film (Plate 4.1). The cord was previously found not to be toxic to *Laminaria* gametophytes (Holt, 1984; Dawes, 1987). The cords were soaked in freshwater for several days, changing the water daily to leach out any undesirable substance which might be harmful to *Palmaria* spores. A wooden frame (1 x 0.45m) was soaked in freshwater and seawater for several weeks for the same reasons. Cords were wound on to the wooden frame and placed in a black PVC tank (1.25 x 0.61m). The tank was filled with 2 micron filtered seawater and left in the constant temperature room overnight to stabilize the temperature. The seawater was enriched with Provasoli's medium half concentration with 2 mg/l germanium dioxide which give the best growth rate of *Palmaria* sporelings (Chapter One). The temperature of the constant temperature room was set at 10^0C and the photoperiod was set at 12:12 h (light:dark) in 1987 and 16:8 h in 1988. Green light with photon irradiance between 30 and 45 μmol $m^{-2}s^{-1}$ was used throughout the seeding programme.

The method of obtaining spores was described in Chapter One. Spore suspensions were poured evenly on to the frame. No attempt was made to count spore number but spore density was judged visually by the depth of the red colour of the suspension. As the viability of the spores could not be guaranteed, seeding was repeated for the next one or two days. By this method, hopefully, sufficient spores would germinate. A week later two small submersible pumps were placed diagonally in the tank to provide gentle water movement. It was thought that this would enhance nutrient uptake by the sporelings, promoting faster growth (Chen and Taylor, 1980) and stimulate the

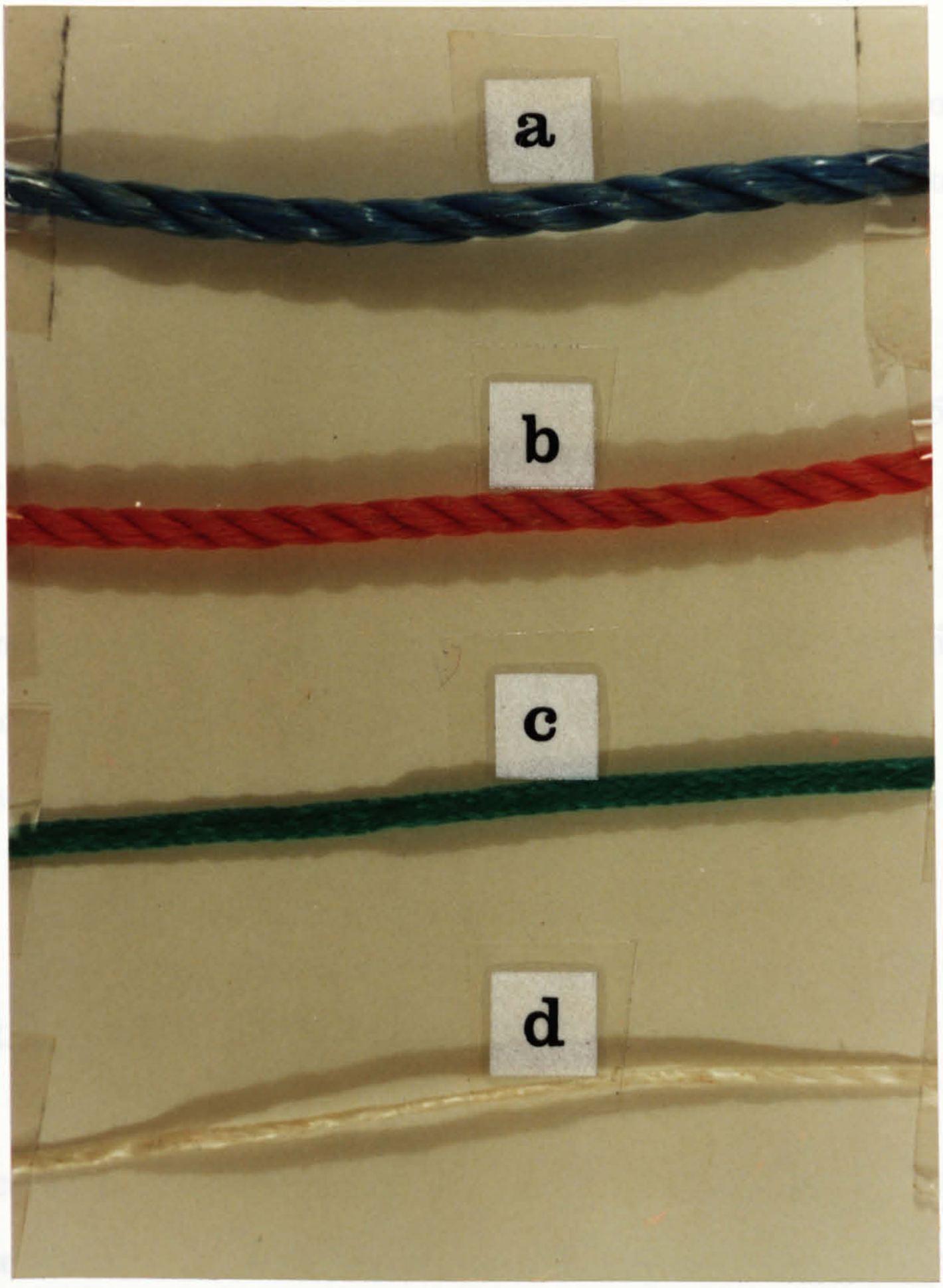


Plate 4.1. 4 different type of cords used for growing ***Palmaria*** from spores and vegetative fragments,

a) 6mm polypropylene cord,

b) 6mm polyethylene cord,

c) 3mm braided polyethylene cord,

d) synthetic fibre film.

formation of stronger holdfast attachment. However, previous work on cultivation of ***Laminaria*** on rope by Holt (1984) did not find any significant improvement in attachment of ***Laminaria*** gametophytes when the medium was aerated during incubation.

Germinated ***Palmaria*** tetraspores will produce about 50% male and 50% female plants (van der Meer and Chen, 1979). There was no attempt to fertilize the female plants. Seeded frames were incubated in the constant temperature room for about 3 weeks and transplanted to the sea as soon as possible to avoid contamination by unicellular and filamentous green algae and deteriorating culture conditions. However this was not always possible as the state of the sea often dictated the seeding program.

4.3. Growing fragments of *Palmaria* thalli on the cords

Cords used for the purpose must be soft and fibrous in order not to injure the plant inserted between the strands. For the purpose 6mm fibrous polypropylene cord (Plate 4.1) was used because it offered a firm secure grip but was at the same time gentle to the plant. Fragments of ***Palmaria*** plants about 0.5 to 1.5g each were inserted about 2cm apart with the help of "fit" (Plate 4.2). Fit is a stainless steel groove with wooden handle which was inserted between the strands of the cord. Fragments of ***Palmaria*** were placed in the groove and the fit was removed leaving the fragments in placed between the strands. Cords with fragments of ***Palmaria*** plants were tied to the horizontal rope in a similar way to the seeded cords mentioned earlier.

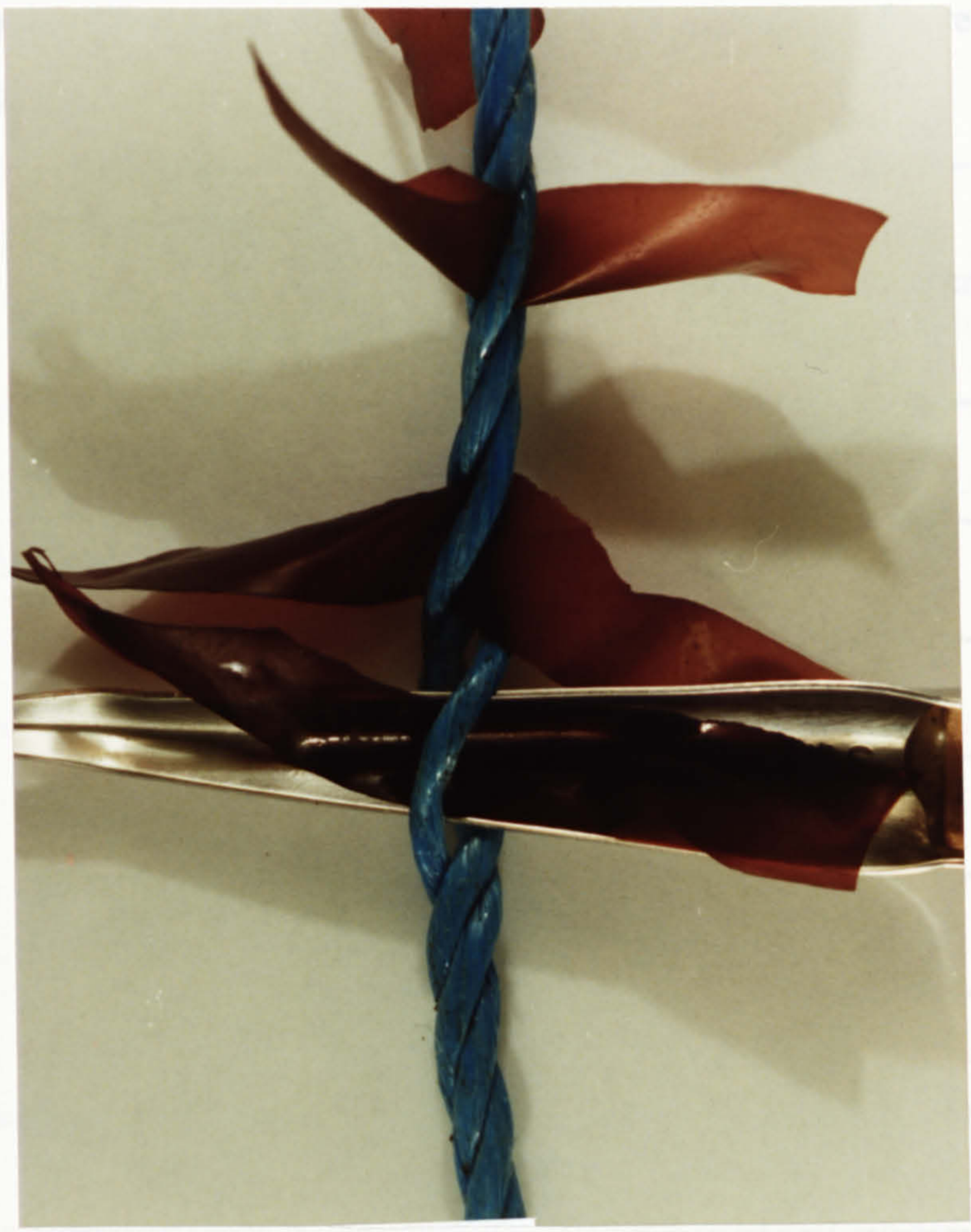


Plate 4.2. "Fit" used to insert vegetative fragments of ***Palmaria***
between the strands of the cord.

Results

4.1. Seeding *Palmaria* spores on to the cords

During the months of January to April 1987 a total of 56 vertical cords were transplant to the sea (Table 4.1).

Date of seeding	Group of cords	Date of transplant	Average sporelings/linear cm at transplant
24-26/11/86	A	26/1/87	no count
1-5/12/86	B	26/1/87	no count
28-31/1/87	C	20/2/87	18.6 ± 6.2
29/1/87-1/2/87	D	20/2/87	17.2 ± 5.1
22/2/87	E	24/3/87	26.2 ± 6.8
23/2/87	F	24/3/87	18.0 ± 5.5
26-27/3/87	G	28/4/87	17.2 ± 3.6
26-27/3/87	H	28/4/87	18.0 ± 5.0

Table 4.1. Time of seeding, date of transplant and average number of sporelings per linear centimetre of cord during 1986-1987. Each group consist of 7 cords.

Each group consisted of seven vertical cords which were tagged. All groups were seeded on to the 6mm smooth polyethylene cord. The vertical cords were checked in May 1987. All the vertical cords were covered with a dense coat of diatoms. When the cord was washed, small *Palmaria* plants about 1 to 2cm in length were growing

underneath together with the Laminariales juveniles about 4 to 6cm in length. Exceptions were the two groups of cords transplanted on 28/4/87 where all of them were bare. Quite a number of young ***Palmaria*** plants were detached and lost while checking the system, so it was decided that no further inspection would be carried out until they are ready for harvest.

On 3/10/87 all the cords were harvested and checked individually whenever possible. Vertical cords transplanted on 26/1/87 (groups A and B) were completely covered with Laminariales plants and none had any ***Palmaria*** growing on them. It must be noted that groups A and B were kept in the constant temperature room for about two months because the sea condition was too rough to transplant the cords. The conditions in the incubation tanks were poor, unicellular and filamentous green algae had started to establish themselves during this period. Perhaps because of the long period of incubation, the sporelings were unable to form strong holdfasts and adapt to the rough sea conditions. The combination of the above factors perhaps could have explained why there were no ***Palmaria*** plants growing on the cords.

Group C, D and E, F were transplanted on 20/2/87 and 24/3/87 respectively had some ***Palmaria*** plants growing on them. The number of ***Palmaria*** plants growing on the cords was very low and they were patchy. Some of the vertical cords were badly tangled with each other and with the horizontal rope so they had to be cut off and lost. The remaining vertical cords were covered with Laminariales plants. A thorough inspection was carried out on those cords and the results was presented on Table 4.2. Most of the cords were covered with Laminariales particularly ***Laminaria saccharina, Alaria esculenta*** and small number of ***Saccorhiza polyschides.*** A closer inspection among the masses of Laminariales revealed that there were some ***Palmaria*** growing with them. At first it seemed that the ***Palmaria*** plants were growing on the ***Laminaria*** holdfast but when the holdfast was cleared very carefully the ***Palmaria*** plants were seen actually growing on the vcord (Plate 4.3).

DEPTH FROM SURFACE (m)	NUMBER OF *PALMARIA* PLANTS PER CORD (WEIGHT IN BRACKETS)									
	C1	C2	C3	C4	D1	D2	D3	D4	E3	F1
1.0	-	3 (5.56)	-	-	-	1 (0.68)	-	-	-	-
2.0	5 (10.97)	2 (30.6)	1 (3.30)	2 (2.79)	1 (3.47)	1 (10.42)	1 (1.64)	1 (0.64)	1 (3.02)	-
3.0	4 (43.3)	1 (35.84)	2 (6.38)	2 (56.58)	1 (1.14)	2 (3.78)	1 (24.2)	2 (41.93)	-	3 (11.01)
4.0	-	2 (13.2)	1 (1.02)	2 (3.81)	-	4 (109.58)	2 (59.9)	1 (2.55)	-	-
5.0	5 (16.05)	1 (11.45)	1 (2.83)	2 (4.64)	2 (39.48)		2 (63.62)	-	1 (3.15)	-
6.0	3 (17.37)	-	1 (42.75)	-	-	2 (24.26)	-	1 (21.29)	-	-
7.0	-	1 (3.57)	-	-	-	-	-	2 (16.94)	-	-

Table 4.2. Number of *Palmaria* plants grown from spores and total weight (in brackets) per metre of cord in 1987.

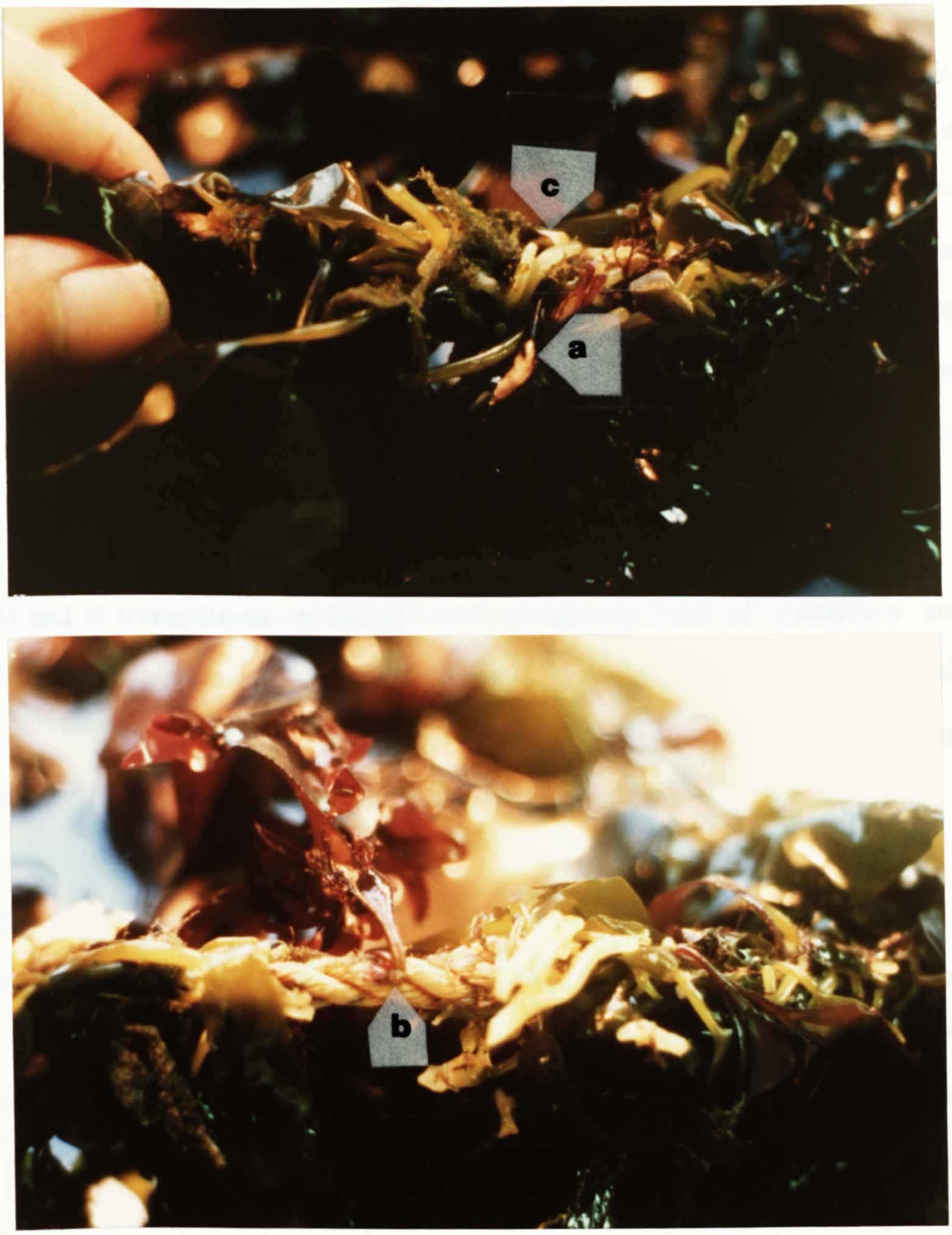


Plate 4.3. ***Palmaria*** plant growing on the cord,

a) before ***Laminaria*** holdfast was cleared,

b) after ***Laminaria*** holdfast was cleared showing,

Palmaria holdfast attaching directly on the cord,

c) ***Laminaria*** holdfast encircling the cord.

Most of the ***Palmaria*** plants that remained on the cords were attached in this way. When the ***Palmaria*** holdfast was detached from the holdfast, the base of the holdfast was seen to follow the contour of the twine of the cords (Plate 4.4). The ***Palmaria*** plants that grew on the cords appeared to be healthy with a deep red colour and a palmate shape (Plate 4.5). The length ranged between 6cm and 38.5cm and the average length was 19.6cm (Fig. 4.3). The weight ranged from 0.12gm to 63.5gm with average weight of 10.8gm (Fig. 4.4). Most of the plant were small and weighed between 0.12 and 6.56g. The range of length and weight of ***Palmaria*** was great.

Group G and H transplanted on 28/4/87 were completely bare of ***Palmaria*** and Laminariales. This was probably because at this time conditions were favourable for colonisation by diatoms (Holt, 1984) which probably killed ***Palmaria*** sporelings and prevented the settlement of Laminariales spores.

In 1988, 4 groups of cords were transplanted on the 17th of February. Group 1, 2 and 3 were seeded while the 4th group were not seeded and served as the control. Group 1 was 6mm fibrous polypropylene, group 2 was 3mm braided polyethylene, group 3 was synthetic fibre film inserted into 6mm smooth polyethylene and the group 4 were plain 6mm fibrous polypropylene cord. Each group consisted of seven vertical cords.

Unfortunately the system broke loose on 23/6/88 and had to be brought in. As the system also contained vertical cords with vegetative fragments of ***Palmaria***, it was very difficult to check all the vertical cords. Therefore the seeded vertical and the control cords were inspected visually without making any record of the number of ***Palmaria*** plants.

The results were very similar to those in 1987. ***Palmaria*** plants grew sparsely amongst the masses of Laminariales in group 1 (6mm fibrous polypropylene) and group

Plate 4.4. Detached ***Palmaria*** holdfast from cord (arrow) showing the contour of the twines of the cord.

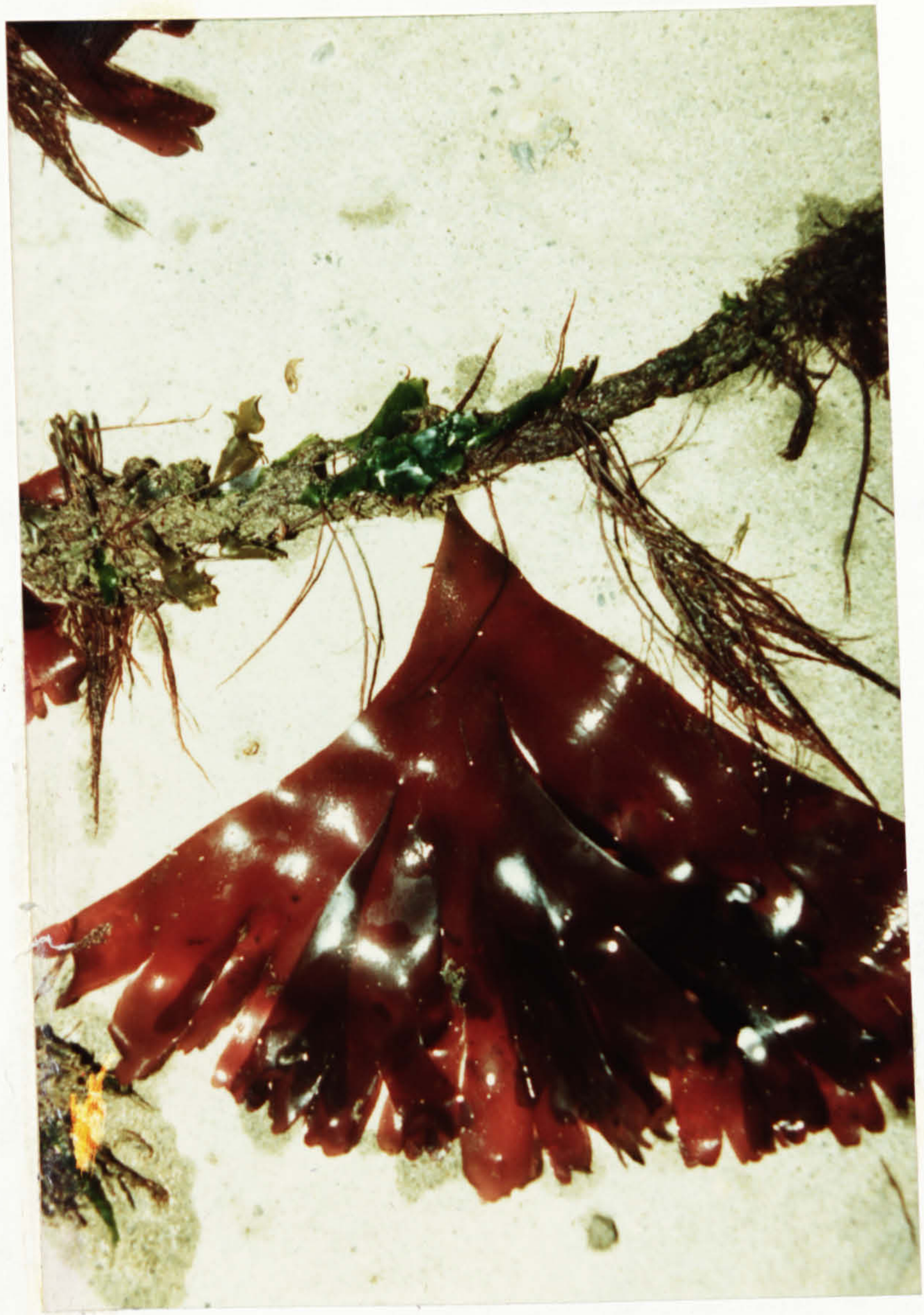


Plate 4.5. Deep red palmate shaped ***Palmaria*** plant growing on the cord from spores.

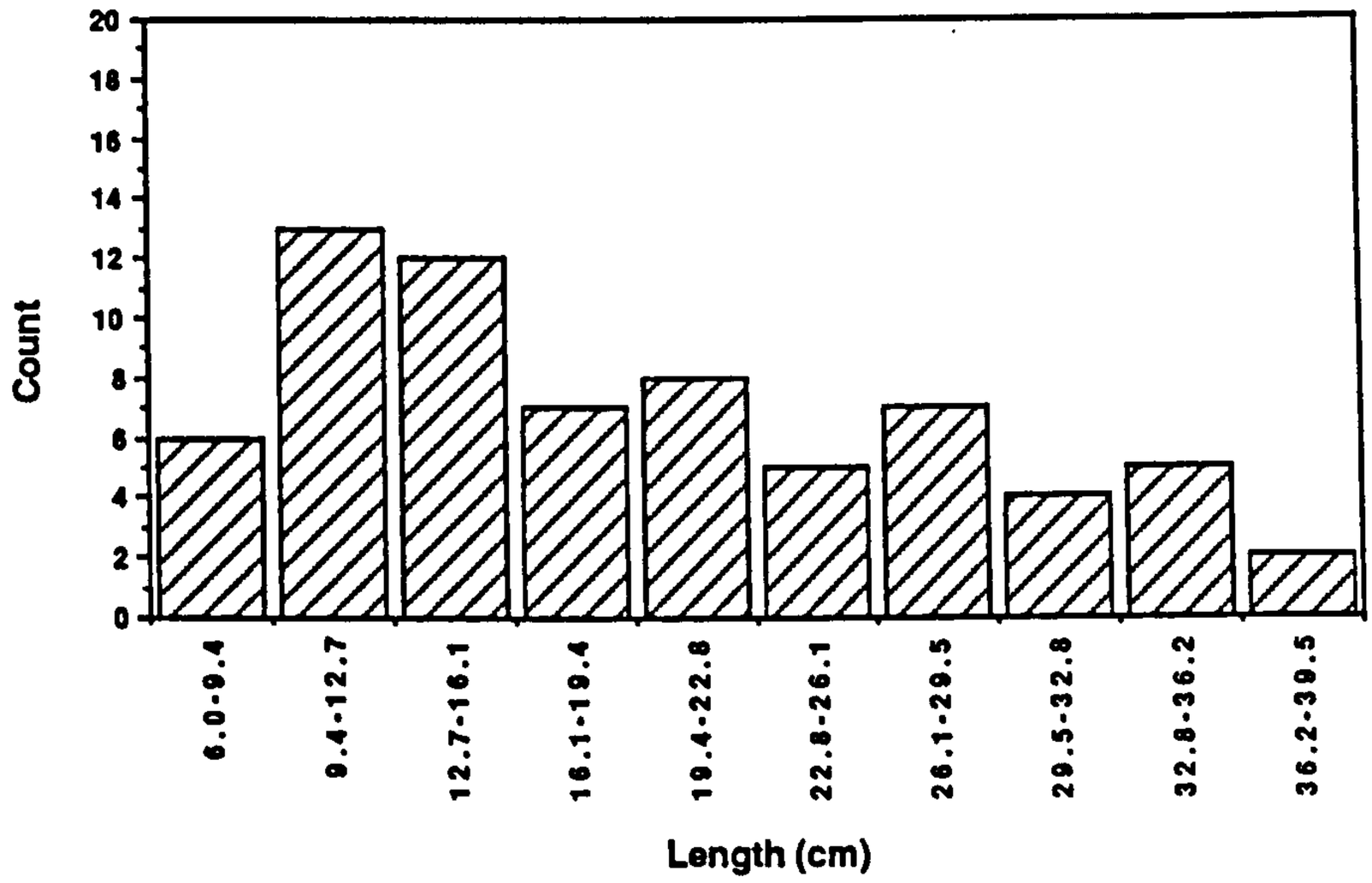


Fig. 4.3. Length frequency distribution of ***Palmaria*** plants growing on cords in 1987.

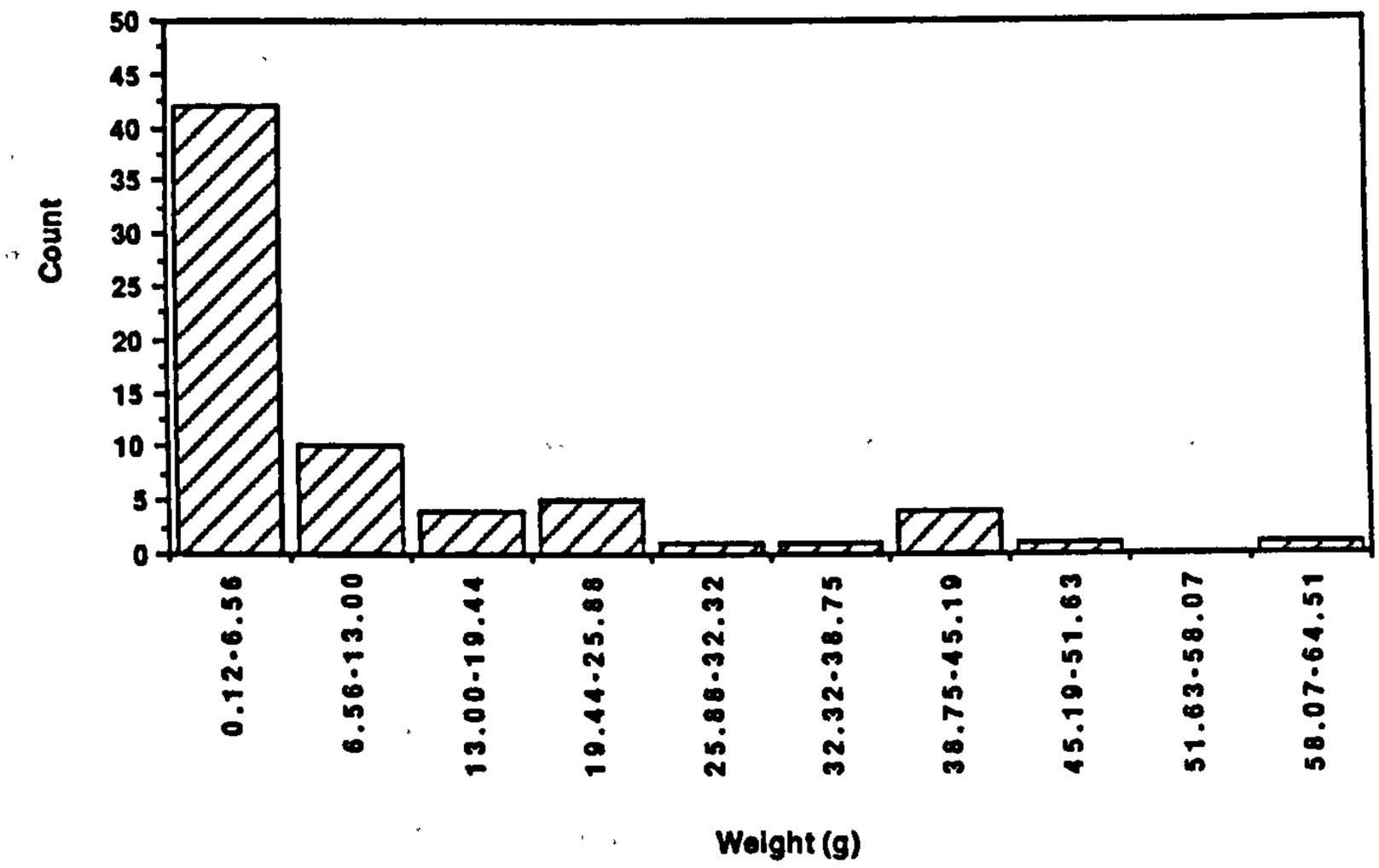


Fig. 4.4. Weight frequency distribution of ***Palmaria*** plants growing on cords in 1987.

3 (synthetic fibre film). In group 3, there were quite a number of small *Palmaria* plants, the size ranging between 6 and 12cm long growing on the synthetic fibre film without "reinforcement" from Laminariales holdfast. Also in group 3, there was high number of Laminariales plants growing directly on to 6mm cords. Five of the group 2 cords(3mm braided polyethylene) were completely bare while the other two were lost.

Unfortunately only two out of seven in the group 4 (control) were recovered. The cords were covered with Laminariales and only 3 *Palmaria* plants were found, on one of the cords.

4.2. Growing vegetative fragments of *Palmaria* on the cords

Cords with pieces of *Palmaria* thalli were transferred to the sea on 20/1/88 and 17/2/88. A total of about 30 vertical cords were transplanted. On 23/6/88 the same system where the seeded cords were placed broke loose and became entangled and had to be brought in.

Out of 30 cords with fragments of *Palmaria* transplanted, 16 cords were recovered. The rest were either badly tangled or lost. Most of the cords were covered with Laminariales which sometimes even attached themselves on to *Palmaria* thalli. It is possible that some *Palmaria* plants broke off from the cords under the weight of Laminariales.

Most of the *Palmaria* plants that remained attached to the cords had marginal proliferations which held the plants in place. Quite a number of *Palmaria* plants were lost while harvesting, evidenced by the small pieces that remained between the cord. Some of the original thalli also died: this was apparent from the bare cord. There were also numerous small plants attached directly on to the cord, possibly from natural

settlement. This is likely because some of the original thalli were sporing tetrasporophytes. The ***Palmaria*** plants, as mentioned previously, were healthy with deep red colour with no apparent differences in appearance between plants growing near the surface and the bottom.

Details of the ***Palmaria*** plants that remained on the cords at various depths are presented in Table 4.3. The size of the ***Palmaria*** plants ranged from few centimetres to 45 centimetres in length. Marginal proliferations that originated from the original thalli developed to sizes ranging from small to big plants. No clear pattern regarding the growth of ***Palmaria*** with depth can be made because of the plants being detached during harvesting, decaying thalli and also because of the influence of fouling Laminariales mentioned previously. Fig. 4.5 shows mean wet weight of ***Palmaria*** and fouling Laminariales that remained on cords with depth. However based on the ***Palmaria*** plants that remained on the cords, the minimum mean wet weight per metre of the cord was 14.70g and the maximum mean wet weight was 49.39g. The overall mean wet weight per 7m of cord was 34.69g. This value was very low compared to the mean wet weight of the fouling Laminariales per 7m of cord which was 321.50g.

Fig. 4.6 shows the weight of individual ***Palmaria*** plants that detached from the cords during harvesting. Plants sizes ranging from less than 2g to 129g. Since ***Palmaria*** thalli are fragile, it is likely that the bigger plants got detached from the cords. In fact a wide range of sizes was well represented in numbers.

DEPTH FROM SURFACE (m)	NUMBER OF *PALMARIA* PLANTS (WEIGHT IN BRACKETS)															
	S 1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
1.0	1 (0.59)	48 (95.62)	-	-	-	-	29 (90.63)	210 (130.48)	24 (20.39)	-	46 (36.13)	20 (148.64)	31 (57.43)	14 (84.72)	2 (28.95)	14 (22.72)
2.0	12 (3.52)	100 (157.40)	-	-	-	5 (3.81)	27 (21.24)	155 (131.06)	20 (39.88)	12 (32.65)	47 (61.77)	-	54 (218.03)	18 (10.31)	-	27 (99.84)
3.0	17 (65.0)	12 (6.13)	33 (21.10)	-	-	7 (26.36)	39 (28.03)	26 (51.16)	24 (160.65)	22 (16.87)	23 (51.72)	21 (21.76)	17 (62.37)	13 (38.27)	15 (101.73)	34 (23.19)
4.0	23 (20.27)	6 (5.45)	40 (13.80)	19 (14.20)	-	12 (65.64)	51 (67.62)	30 (15.04)	6 (60.26)	12 (53.68)	34 (48.87)	14 (20.84)	-.	14 (145.45)	20 (30.14)	14 (10.37)
5.0	28 (8.92)	15 (4.30)	53 (65.88)	60 (120.33)	15 (191.08)	22 (122.75)	28 (19.70)	42 (27.00)	8 (19.47)	-	27 (27.91)	21 (88.97)	6 (131.28)	24 (58.84)	30 (58.69)	30 (16.15)
60	7 (2.62)	27 (17.95)	112 (198.76)	50 (65.04)	15 (105.28)	4 (69.90)	16 (7.30)	9 (3.60)	-	-	23 (78.26)	40 (135.55)	5 (21.50)	20 (10.49)	16 (31.46)	18 (13.23)
7.0	10 (25 6)	5 (41.09)	-	-	13 (10.42)	1 (4.02)	14 (60.62)	11 (13.11)	-	-	8 (3.60)	-	4 (35.40)	6 (40.26)	2 (9.55)	11 (17.20)

Table 4.3. Number of ***Palmaria*** plants grown from fragments on cords between 20/1/88 and 17/2/88 to 23/6/88 and total weight (in brackets).

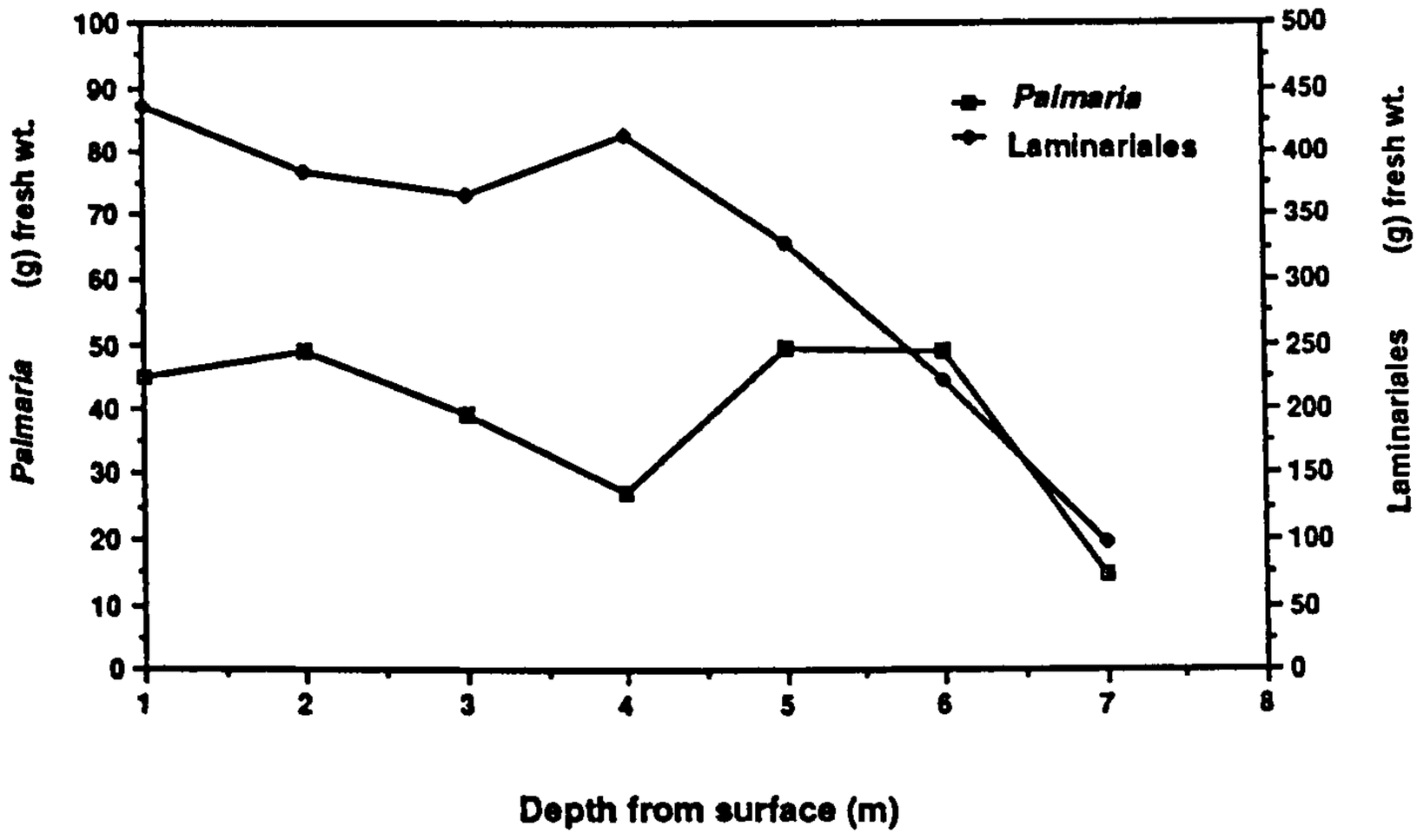


Fig. 4.5. Mean weight per metre of cord of *Palmaria* plants from fragments and fouling Laminariales on 16 cords with depth in 1988.

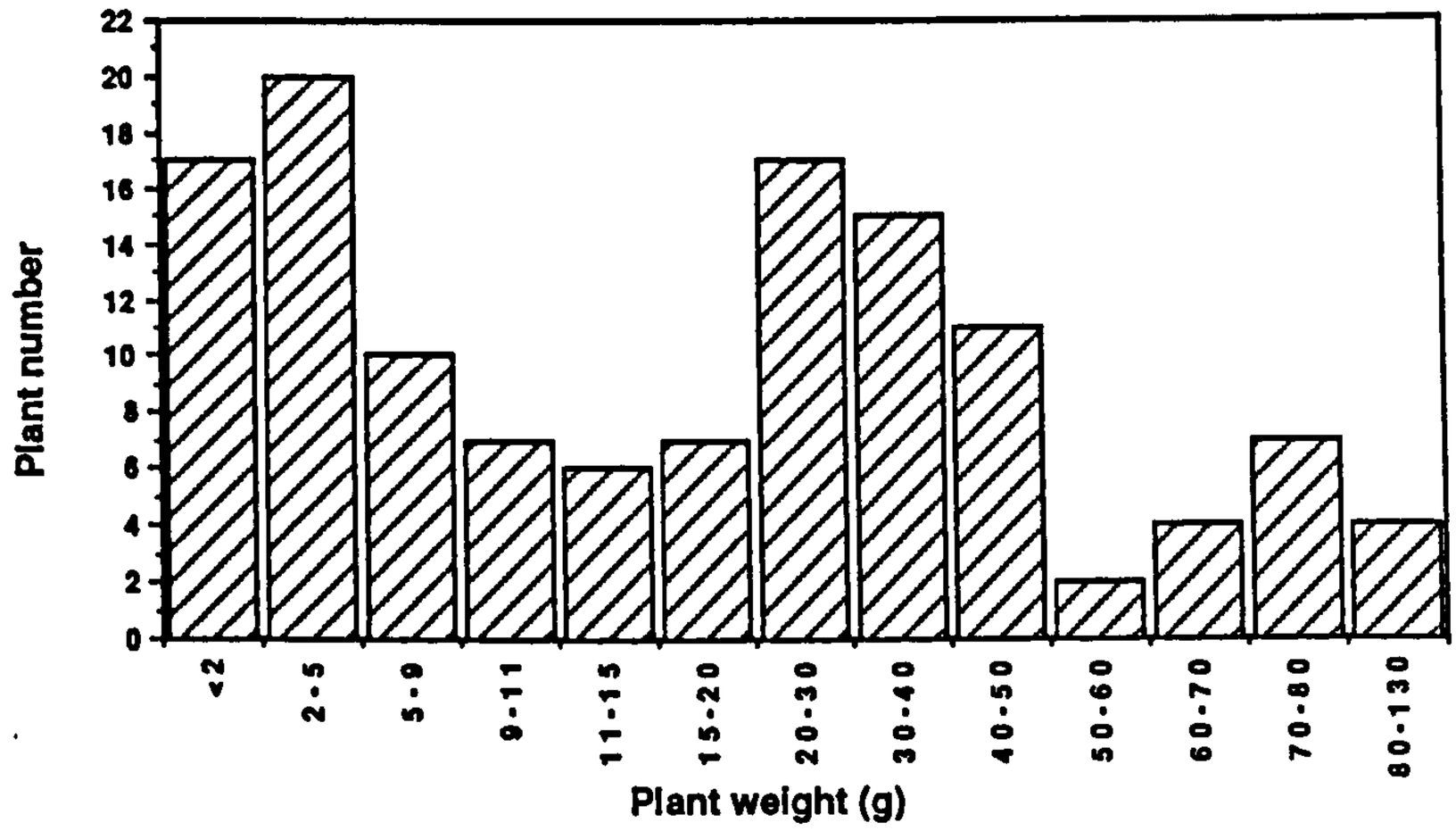


Fig. 4.6. Weight frequency distribution of ***Palmaria*** plants developed from fragments previously inserted into cords, which became detached during harvesting in 1988.

Discussion

When the seeded cords were transplanted to the sea, the mean number of sporelings per centimetre of cord were between 17.0 and 26.2. Since it was difficult to determine precisely whether the sporelings were male or female under disecting microscope, let us assume that about half of them were male. If the female sporelings were not fertilized and remained stunted (van der Meer and Todd, 1980) that means about 8 male sporelings had the potential to grow into adult plants per centimetre or 800 plants per metre of cord.

But when the cords were harvested, the number of adult ***Palmaria*** (presumably male) that remained were very low and far apart. It has been mentioned earlier that most of ***Palmaria*** plants that remained on the cords were "reinforced" by Laminarian holdfasts and to a certain extent some small plants growing between Laminariales plants. There are several possibilities for the low number of ***Palmaria*** plants remaining on the cord. Perhaps the inability of the ***Palmaria*** holdfast to secure firmly on to the cord is one of them. Unlike Laminariales holdfasts which are big and able to encircle the cord (Plate 4.3c) (Dawes, 1987) to provide a secure attachment, ***Palmaria*** plants have small holdfasts, less than 10mm in diameter (Sparling, 1961; Guiry, 1976) and only attach to a small area of the cord. While it is also true that ***Palmaria*** plants that grow on Laminariales only occupy a s mall area of the stipe the attachment is more secure and to a certain degree ***Palmaria*** plants are sheltered from water current. In the rope system however the situation is quite different. It is more exposed to water currents and wave action and the cord shape or structure will change according to the sea conditions. In the sea, the vertical cords will flex, twist and untwist due to wave action and water current, thus weakening the holdfast attachment.

In the rope system, when ***Palmaria*** plants are small, they are less affected by water current, but as they grow, the increased surface area of the blade also increases the drag force caused by the water current. The conditions at Bay Fine are exposed and quite often the sea is rough. There were also water currents up to 2.9 ms^{-1} during spring tides. Excessive water motion may result in mechanical damage and detach plants from the substrata especially in smaller species (Mshigeni, 1976; Holt, 1984).

Another explanation for why the number of ***Palmaria*** plants that grew on cords was low and patchy could be the presence of colonial diatoms. It has been mentioned that during spring and early summer the cords were covered by a dense growth of diatoms. Diatoms have been known to smother macroalgae particularly in rope culture (Holt, 1984; Dawes, 1987; Sylvester and Waaland, 1983). Juveniles of brown seaweeds such as ***Laminaria*** and ***Alaria*** can cope with fouling diatoms better than ***Palmaria*** which is smaller and slower growing (Holt, 1984). Dense colonies of diatoms deprive ***Palmaria*** of much needed light and nutrients. Apart from that there is also a possibility that diatoms might have harmed ***Palmaria*** sporelings directly by secreting inhibiting substances although it is difficult to prove this in the field. Tests carried out in the laboratory showed that diatoms have a negative effect on some of the red seaweeds (Huang and Boney, 1984, 1985).

It has been observed that ***Palmaria*** plants that remained on the cords within the same seeding group have wide size variation. The chances that smaller plants originating from natural colonization was very low because the seeded cord transplanted on 26/1/87 and group 4 unseeded cords transplaneted on 17/2/88 have none or very small number of ***Palmaria*** plants growing on them. Therefore it is most likely that small ***Palmaria*** plants were originating from the same spores but were stunted due to a heavy canopy of Laminariales. Heavy growth of Laminariales plants probably reduced the light received by ***Palmaria*** resulting in reduced growth and development.

It is also worth mentioning that ***Palmaria*** plants had remained healthy when harvested on 3/10/87 without showing any sign of nitrogen starvation and pigment destruction. Nitrogen starved ***Palmaria*** normally lacks red pigment and is greenish in colour (Morgan ***et al.***, 1980a). Laminariales plants that grew on the cords also showed minimal deterioration except that most of the blades were encrusted with bryozoans. The wild population of ***Palmaria*** in the surrounding area also remained healthy. In contrast Dion and Delépine (1981) observed at Roscoff, France that after June the growth of ***Palmaria*** decreased, the thalli lost their pigmentation, were covered with numerous bladelets and hyaline hairs and by mid July and August all the erect thalli had disappeared. The mean nitrogen level between the month of May and August taken 5km offshore from Bay Fine was below 1ug at/l (Fig. 4.7) (Graziano, 1988). However the study area was shallow with ample of water movement perhaps improving the efficiency with which thalli absorb nutrients even though the ambient nutrients were very low. Natural seaweeds are often described as more luxuriant in turbulent areas (Conover, 1968).

The results of ***Palmaria*** grown from fragments of thalli were better than those grown from spores. More plants were recovered per meter of cord. Perhaps the fragments of ***Palmaria*** were able to withstand fouling diatoms better than sporelings and there was no holdfast problem. Perhaps natural germination that occurred on the cord (Braud and Perez, 1978) also contributed to the high number and variable size of plants recovered.

The problem which was common in both systems was fouling by unwanted species of seaweeds such as Laminariales which in both cases masked the results of growing ***Palmaria*** from spores and from fragments. Similar problems were observed elsewhere (Guo-zhong ***et al.***, 1984; Mumford, 1977; Hanic and Pringle, 1978). Due to their massive size and fast growth rates, kelps can overgrow ***Palmaria***, shielding ***Palmaria*** from receiving enough light, competing for growing space and

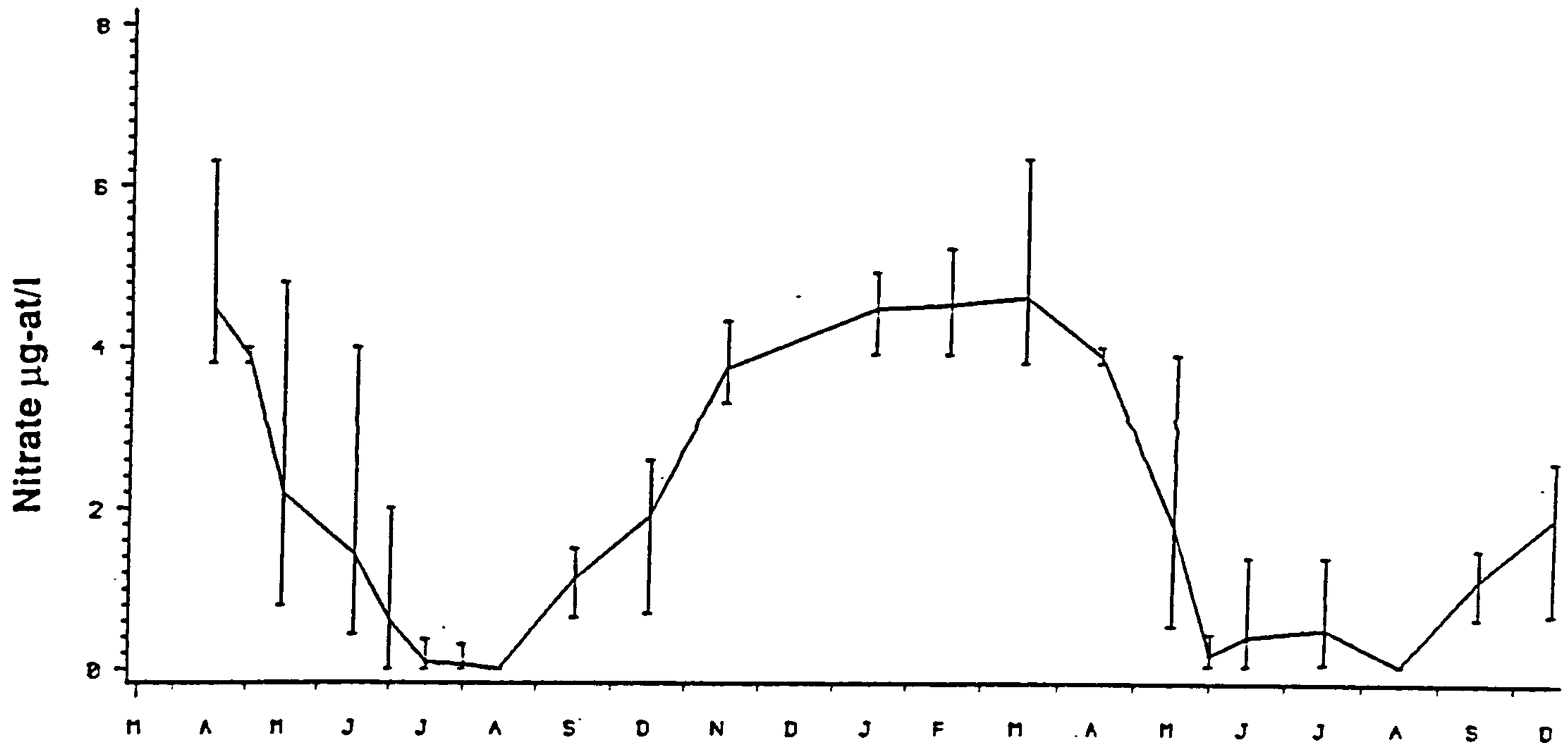


Fig. 4.7. Seasonal variation of nitrate (µg-at/l) 5km off Port Erin (Graziano, 1988).

harming the rope system structure which was not designed to support the massive weight of Laminariales.

GENERAL DISCUSSION

The ultimate aim of any mariculture programme is to grow a product of quality acceptable to the consumer at a reasonable price and with a sufficiently low production cost for the venture to be economically viable. Prospects and problems of cultivating ***Palmaria palmata*** vegetatively in onshore culture tanks, growing from spores and fragments on cords in the sea have been discussed in Chapters Two and Four.

It has been mentioned in Chapter Two that cultivating ***Palmaria*** vegetatively in onshore tanks is not economically viable in Port Erin, Isle of Man because of the high production costs. The cost is mainly for pumping seawater, collection of plants and labour for maintaining the culture system.

Demand for seawater, thus the cost, can be reduced by adopting better culture techniques such as control of pH, addition of CO_2 and pulse fertilization. The production costs can be reduced further by growing ***Palmaria*** in a floating cage in the sea (Davis, 1980) but the sea around Port Erin and around the Isle of Man in general is often too rough to maintain such structures. In tank culture new plants have to be brought in periodically to replace those harvested. This can be avoided by harvesting the older thalli and regrowing the tips which have faster growth, as shown in Chapter Three, but the process is laborious and impracticable in a big scale culture. The inability of ***Palmaria*** to produce marginal proliferations at some time of the year perhaps is a single decisive factor why this method of cultivation is not suitable for this species in Port Erin, Isle of Man.

Cultivation of ***Palmaria*** in the sea from spores and vegetative fragments suffers from one common problem, that is fouling by unwanted seaweeds, particularly Laminariales. Because they have fast growth, they can overgrow ***Palmaria***,

shielding it from getting sufficient light, and damaging the rope structure which was not designed to support the massive weight of the Laminariales. Since fouling seaweeds respond to the same growing conditions as ***Palmaria*** they are very difficult to eliminate. Transplanting the seeded cords either from spores or fragments during the time when there is less settlement of spores of unwanted seaweeds seems unlikely to be successful because of the similarity in sporing periods. Methods of desiccating the seeded cords or nets periodically to reduce fouling seaweeds which is routinely employed in ***Porphyra*** culture (Miura, 1975) cannot be employed because ***Palmaria*** sporelings are very delicate. However it is possible to apply this technique to the cords seeded with ***Palmaria*** fragments. ***Palmaria*** fragments in general are tougher than sporelings or the "contaminants" spores, but there is a need for further study to know the duration of desiccation period which is enough to kill the fouling "contaminants" spores without harming the ***Palmaria*** fragments. Setting up the rope culture systems well away from natural population of seaweeds such as in open water could be the other possibility but it can be costly and difficult to inspect the systems regularly. It will take sometime before any of the above suggestions becomes reality. At present one of the possibilities is to remove the contaminants by hand which not only involves a lot of labour but detaches ***Palmaria*** from the cord in the process.

If fouling by unwanted species of seaweeds can be reduced or eliminated what is the future of growing ***Palmaria*** from spores or fragments on cords in the sea? First let us consider growing ***Palmaria*** from spores. Growing ***Palmaria*** from spores on cords in the sea involves several processes such as collection of sporing materials, obtaining enough spores for seeding, incubation of seeded cords in incubation tanks and transplantation of seeded cords to the sea. The length of the process will be a disadvantage because it involves time and money and also possible problems might arise at each stage.

At present there are several problems of growing ***Palmaria*** from spores. These problems are variation in the number of spores shed, spore viability and low percentage of germination. As a result not all the seeded cords become covered with ***Palmaria*** sporelings, thus allowing spores of unwanted seaweeds to settle and compete with ***Palmaria*** sporelings. Good germination will result in too many sporelings growing on the cords which leads to stunted growth as shown in Chapter One. Therefore there is a need to study the basic spore biology and physiology and find a way to improve spore germination.

Another problem with the present technique is that only one side of the cords can be seeded because of the difficulties in getting spores to settle. This again allows the spores of unwanted seaweeds to settle and grow on the other side of the cords. It was found in Chapter One that the inability of the spores to settle on the substrate is probably one of the reasons for low percentage of germination. Chamberlain (pers. comm.) suggested that coating the cord with a layer of polylysine, a long chain polymer which helps ***Palmaria*** spores to settle on the cord.

It has been mentioned in Chapter Four that the area in which this study is conducted is often very rough with a strong water current which probably washed off the delicate ***Palmaria*** sporelings or plants from the cord. ***Palmaria*** plants unlike some species of seaweed attach to substrate by discoidal rather than rhizoidal holdfast which has the ability to anchor firmly to the fibres or twines of the cord. The inability ***Palmaria*** holdfast to form a secure attachment on the cord is probably the reason why the sporelings are easily washed off by strong water current. A sheltered location with moderate or little water movement perhaps is more suitable for growing ***Palmaria*** from spores. But then the plants may suffer nutrient shortage in summer. This problem can be overcome by applying fertilizer on the culture site (Tseng, 1981a).

Fouling by diatoms is known to have a direct or indirect detrimental effects on ***Palmaria*** sporelings. Fouling by diatoms can be avoided if the cords are transplanted earlier or the cords are lowered in deeper water and raised to the original position when the bloom is over (Dawes, 1987).

An alternative to growing ***Palmaria*** from spores is by growing from fragments. Initial results suggested that inserting fragments into the cords and transplanting to the sea have many advantages over growing from spores. The process of growing from fragments is simpler. However the present technique is rather laborious and time consuming but in the future efficient methods of inserting fragments through mechanization or by other means could reduce the cost and time.

Initial results indicated that fragments can withstand fouling by algal blooms and rough seas better than young sporelings. By growing ***Palmaria*** from fragments, crops can be obtained through marginal proliferations and also from spores if sporing plants are used as original seed stock. There is also a possibility that leaving parts of the thalli on the cord during harvesting will enable the remaining thalli to produce the next generation of plants in the next growing season thus reducing the need to seed the cords every year. This is difficult to achieve because of the fouling problem. Therefore growing ***Palmaria*** from fragments on cords seems to be a better technique than growing from spores or growing vegetatively in onshore tanks. If growing ***Palmaria*** from fragments is adopted, there is an urgent need to overcome the fouling problem, determine depths for best growth and develop better methods of inserting fragments into the cords.

The conclusion from this study is that it is unlikely that ***Palmaria*** can be cultured successfully from spores or fragments on cords in the sea around the Isle of Man at present because of low percentage of germination, inability to form secure attachment on to cords, and inability to compete with the opportunistic species such as Laminariales

which attach better, grow faster, and persist better in the rough sea conditions.

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APPENDIX 1

Growth media formulation based on (McLachlan, 1973) except IWA-SWII.

	Concentration per liter of medium				
Additive	PES	F/2	SWM-3	VS	*IWA-SWII
KNO_3	-	-	-	-	.71mM
$NaNO_3$	0.66mM	0.88mM	1.25mM	0.5mM	-
KH_2PO_4	-	-	-	-	0.033mM
Na_2glycero-phosphate	25 µM	-	-	-	0.034mm
NaH_2PO_4	-	-	-	30 µM	-
Na_2SiO_3	-	0.081mM	0.2mM	-	-
B_{12}	1.6 µg	0.5 µg	-	-	-
Biotin	0.8 µg	0.5 µg	-	-	-
Thiamin.HCl	20 µg	100 µg	-	-	-
Vitamin Soln.	-	-	S-3	-	-
Fe.EDTA	**7.2 µM	-	2.0 µM	-	0.65 µM
$Fe_2(SO_4)_3$	-	-	-	1 µM	-
$MnCl_2$	-	-	-	0.1 µM	-
Trace Metal Soln.	P-11	F/2	TMS-1	-	-
Tris	0.66mM	-	1.5mM	-	4.1mM
Na_2.EDTA	-	-	-	10 µM	-

* Formulation based on(Iwasaki, 1961)

** (ammendment made by Grandy, 1984)

Continue

Trace metals solution

Element	Concentration µM /liter of medium P-11	F/2	TMS-1
Zinc	0.8	0.08	35
Mangenese	7.3	0.9	10
Molybdenum	-	0.03	5
Cobalt	0.17	0.05	0.3
Copper	-	0.04	0.3
Iron	1.8	11.7	2.0
EDTA	26.9	11.7	48
Boron	185	-	400

Vitamins Solution

Additive	S-3 (conc./ liter of medium)
Thiamine.HCl	0.5mg
Nicotinic acid	0.1mg
Ca.Pantothenate	0.1mg
P-Aminobenzoic acid	10.0 µg
Biotin	1.0 µg
i-Inositol	5.0 mg
Folic acid	2.0 µg
B_{12}	1.0 µg
Thymine	3.0mg

APPENDIX 2

VEGETATIVE GROWTH RATE (WEIGHT) OF ***P. palmata*** GROWN IN THE ONSHORE TANKS.

TANK NUMBER: CONICAL TANK I (CONTINUOUS FLOW SYSTEM)

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
26/6/86 - 5/7/86	1493	1844	9	0.0234	54.5
5/7/86 - 12/7/86	1844	1880	7	0.0027	27.5
12/7/86 - 14/7/86	1880	1895	2	0.0040	1.1
14/7/86 - 19/7/86	1595	1847	5	0.0293	11.9
19/7/86 - 26/7/86	1320	1438	7	0.0122	18.8
26/7/86 - 2/8/86	1438	1696	7	0.0236	21.8
2/8/86 - 9/8/86	1526	1808	7	0.0242	35.1
9/8/86 - 16/8/86	1227	1503	7	0.0290	28.0
16/8/86 - 25/8/86	1150	1519.5	9	0.0310	48.7
25/8/86 - 30/8/86	CLEANED TANK AND RESTOCK NEW PLANTS				
30/8/86 - 6/9/86	1000	1183	7	0.0240	46.1
6/9/86 - 13/9/86	1000	1080	7	0.0110	62.3
13/9/86 - 20/9/86	1000	1137	7	0.0183	65.3
20/9/86 - 27/9/86	1000	1198	7	0.0258	19.0
27/9/86 - 4/10/86	1000	1256	7	0.0326	16.5
4/10/86 - 11/10/86	1000	1256	7	0.0326	23.5

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANK.

TANK NUMBER: CONICAL TANK 2

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
3/7/86 - 12/7/86	1060	1320	9	0.0244	28.1
12/7/86 - 19/7/86	1320	1604	7	0.0278	13.9
19/7/86 - 26/7/86	1005	1358	7	0.0430	18.8
26/7/86 - 2/8/86	1358	1751	7	0.0363	21.8
2/8/8 - 9/8/86	1509	1617	7	0.0099	35.1
9/8/86 - 16/8/86	1249	1616	7	0.0368	28.0
16/8/86 - 25/8/86	1616	1915	9	0.0189	48.7
25/8/86 - 30/8/86	1915	2147.5	5	0.0229	10.9
30/8/86 - 6/9/86	1000	1155	7	0.0206	46.1
6/9/86 - 13/9/86	1000	1247	7	0.0315	62.3
13/9/86 - 20/9/86	1000	1097	7	0.0132	65.3
20/9/86 - 27/9/586	1000	1182	7	0.0239	19.0
27/9/86 - 29/9/86	CLEANED TANK AND RESTOCK NEW PLANTS				
29/9/86 - 4/10/86	968	1115	5	0.0283	16.5
4/10/86 - 11/10/86	1000	1260	7	0.0330	23.5

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF ***P. palmata*** GROWN IN THE ONSHORE TANKS.

TANK NUMBER: CONICAL TANK 3

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
2/8/86 - 16/8/86	1300	1646	14	0.0169	63.1
16/8/86 - 25/8/86	1646	1938	9	0.0181	48.7
25/8/86 - 30/8/86	1938	2211	5	0.0264	10.9
30/8/86 - 6/9/86	1000	1138	7	0.0185	46.1
6/9/86 - 13/9/86	1000	1109	7	0.0148	62.3
13/9/86 - 20/9/86	1000	1246	7	0.0314	65.3
20/9/86 - 27/9/86	1000	1160	7	0.0212	19.0
27/9/86 - 4/10/86	1000	1262	7	0.0332	16.5
4/10/86 - 11/10/86	1000	1159	7	0.0211	23.5

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: CONICAL TANK 4

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
2/8/86 - 16/8/86	1300	1723	14	0.0201	63.1
16/8/86 - 25/8/86	1723	1968	9	0.0148	48.7
25/8/86 - 30/8/86	1968	2196	5	0.0219	10.9
30/8/86 - 6/9/86	1000	1184	7	0.0241	46.1
6/9/86 - 13/9/86	1000	1100	7	0.0136	62.3
13/9/86 - 20/9/86	1000	1164	7	0.0217	65.3
20/9/86 - 27/9/86	1000	1219	7	0.0283	19.0
27/9/86 - 4/10/86	1000	1285	7	0.0358	16.5
4/10/86 - 11/10/86	1000	1207	7	0.0269	23.5

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: RECTANGULAR 1 (BATCH SYSTEM)

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
20/6/86 - 24/6/86	1143	1249	4	0.0221	22.1
24/6/86 - 27/6/86	1249	1257	3	0.0021	21.0
27/6/86 - 1/7/86	1257	1381	4	0.0235	23.6
1/7/86 - 5/7/86	1381	1445	4	0.0113	22.9
5/7/86 - 8/7/86	1299	1398	3	0.0245	14.6
8/7/86 - 15/7/86	1398	1524	7	0.0123	14.9
15/7/86 - 22/7/86	1524	1696	7	0.0153	19.6
22/7/86 - 26/7/86	1696	1773	4	0.0111	11.1
26/7/86 - 28/7/86	1773	1840	2	0.0185	8.1
28/7/86 - 29/7/86	1680	1714	1	0.0200	0.0
29/7/86 - 30/7/86	1714	1725	1	0.0064	6.3
30/7/86 - 5/8/86	1579	1635	6	0.0058	23.7
5/8/86 - 9/8/86	1635	1699	4	0.0096	18.8
9/8/86 - 11/8/86	1699	1769	2	0.0202	7.8
11/8/86 - 12/8/86	1571	1607	1	0.0226	0.0
12/8/86 - 2/9/86	STOPPED TEMPORARILY				
2/9/86 - 8/9/86	954	1073	6	0.0196	34.6
8/9/86 - 16/9/86	971	1144	8	0.0205	81.0
16/9/86 - 23/9/86	1000	1073	7	0.0101	36.5
23/9/86 - 29/9/86	900	994	6	0.0166	15.6

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: RECTANGULAR TANK 2

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
20/6/86 - 24/6/86	1103	1214	4	0.0240	22.1
24/6/86 - 27/6/86	1214	1226	3	0.0033	21.0
27/6/86 - 1/7/86	1226	1292	4	0.0131	23.6
1/7/86 - 5/7/86	1292	1325	4	0.0063	22.9
5/7/86 - 7/7/86	1325	1369	2	0.0163	4.9
7/7/86 - 8/7/86	1190	1212	1	0.0183	9.7
8/7/86 - 15/7/86	1212	1290	7	0.0089	14.9
15/7/86 - 22/7/86	1290	1373	7	0.0089	19.6
22/7/86 - 26/7/86	1373	1386	4	0.0024	11.1
26/7/86 - 28/7/86	1386	1399	2	0.0047	8.1
28/7/86 2- 29/7/86	1302	1312	1	0.0077	0.0
29/7/86 - 30/7/86	1312	1315	1	0.0023	6.3
30/7/86 - 5/8/87	1240	1255	6	0.0020	23.7
5/8/86 - 9/8/86	1255	1266	4	0.0022	18.8
9/8/86 - 12/8/86	1266	1302	3	0.0093	7.8
12/8/86 - 2/9/86	STOPPED TEMPERORILY				
2/9/86 - 8/9/86	922	1076	6	0.0257	34.6
8/9/86 - 16/9/86	1076	1145	8	0.0078	81.0
16/9/86 - 23/9/86	1000	1073	7	0.0101	36.5
23/9/86 - 29/9/86	900	970	6	0.0125	15.6

APPENDIX 3

VEGETATIVE GROWTH RATE (WEIGHT) OF ***P. palmata*** GROWN IN THE ONSHORE TANKS.

TANK NUMBER: CONICAL TANK 1 (CONTINUOUS FLOW SYSTEM)

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
29/6/87 - 5/7/87	1000	1234	7	0.0300	51.5
5/7/87 - 12/7/87	1000	1260	7	0.0330	61.4
12/7/87 - 19/7/87	1000	1269	7	0.0340	18.4
19/7/87 - 26/7/87	1000	1242	7	0.0310	66.2
26/7/87 - 2/8/87	1000	1248	7	0.0316	19.5
2/8/87 - 9/8/87	1000	1260	7	0.0330	53.7
9/8/87 - 17/8/87	1000	1281	8	0.0310	38.2
17/8/87 - 23/8/87	1000	1145	6	0.0226	19.8
23/8/87 - 30/8/87	1000	1158	7	0.0210	39.0
30/8/87 - 6/9/87	1000	1217	7	0.0281	46.0
6/9/87 - 13/9/87	1000	1245	7	0.0313	33.5
13/9/87 - 20/9/87	1000	1235	7	0.0302	47.0
20/9/87 - 27/9/87	1000	1218	7	0.0282	41.5
27/9/87 - 4/10/87	1000	1194	7	0.0253	36.5
4/10/87 - 11/10/87	1000	1167	7	0.0221	18.8
11/10/87 - 18/10/8	1000	1187	7	0.0245	37.6
18/10/87 - 25/10/8	1000	1171	7	0.0226	21.1

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: CONICAL TANK 2

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
29/6/87 - 5/7/87	1000	1286	7	0.0360	51.5
5/7/87 - 12/7/87	1000	1264	7	0.0335	61.4
12/7/87 - 19/7/87	1000	1282	7	0.0355	18.4
19/7/87 - 26/7/87	1000	1226	7	0.0291	66.2
26/7/87 - 2/8/87	1000	1247	7	0.0315	19.5
2/8/87 - 9/8/87	1000	1291	7	0.0365	53.7
9/8/87 - 17/8/87	1000	1283	8	0.0312	38.2
17/8/87 - 23/8/87	1000	1191	6	0.0291	19.8
23/8/87 - 30/8/87	1000	1208	7	0.0270	39.0
30/8/87 - 6/9/87	1000	1230	7	0.0296	46.0
6/9/87 - 13/9/87	1000	1246	7	0.0314	33.5
13/9/87 - 20/9/87	1000	1224	7	0.0289	47.0
20/9/87 - 27/9/87	1000	1243	7	0.0311	41.5
27/9/87 - 4/10/87	1000	1176	7	0.0232	36.5
4/10/87 - 11/10/87	1000	1182	7	0.0239	18.8
11/10/87 - 18/10/87	1000	1190	7	0.0249	37.6
18/10/87 - 25/10/87	1000	1218	7	0.0282	21.1

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: CONICAL TANK 3

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
29/6/87 - 5/7/87	1000	1259	7	0.0329	51.5
5/7/87 - 12/7/87	1000	1333	7	0.0411	61.4
12/7/87 - 19/7/87	1000	1245	7	0.0314	18.4
19/7/87 - 26/7/87	1000	1297	7	0.0372	66.2
26/7/87 - 2/8/87	1000	1257	7	0.0327	19.5
2/8/87 - 9/8/87	1000	1194	7	0.0253	53.7
9/8/87 - 17/8/87	1000	1315	8	0.0342	38.2
17/8/87 - 23/8/87	1000	1216	6	0.0329	19.8
23/8/87 - 30/8/87	1000	1210	7	0.0272	39.0
30/8/87 - 6/9/87	1000	1290	7	0.0364	46.0
6/9/87 - 13/9/87	1000	1275	7	0.0347	33.5
13/9/87 - 20/9/87	1000	1249	7	0.0318	47.0
20/9/87 - 27/9/87	1000	1292	7	0.0366	41.5
27/9/87 - 4/10/87	1000	1203	7	0.0264	36.5
4/10/87 - 11/10/87	1000	1185	7	0.0243	18.8
11/10/87 - 18/10/87	1000	1199	7	0.0259	37.6
18/10/87 - 25/10/87	1000	1187	7	0.0245	21.1

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: RECTANGULAR TANK 1 (BATCH SYSTEM)

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
29/6/87 - 5/7/87	850	1010	7	0.0246	51.5
5/7/87 - 12/7/87	850	1001	7	0.0234	61.4
12/7/87 - 19/7/87	850	1007	7	0.0242	18.4
19/7/87 - 26/7/87	850	974	7	0.0195	66.2
26/7/87 - 2/8/87	850	986	7	0.0212	19.5
2/8/87 - 9/8/87	850	963	7	0.0178	53.7
9/8/87 - 17/8/87	850	1023	8	0.0232	38.2
17/8/87 - 23/8/87	850	952	6	0.0189	19.8
23/8/87 - 30/8/87	850	979	7	0.0202	39.0
30/8/87 - 6/9/87	850	989	7	0.0216	46.0
6/9/87 - 13/9/87	850	998	7	0.0229	33.5
13/9/87 - 20/9/87	850	981	7	0.0205	47.0
20/9/87 - 27/9/87	850	1000	7	0.0232	41.5
27/9/87 - 4/10/87	850	965	7	0.0181	36.5
4/10/87 - 11/10/87	850	975	7	0.0196	18.8
11/10/87 - 18/10/87	850	973	7	0.0193	37.6
18/10/87 - 25/10/87	850	962	7	0.0177	21.1

CONTINUE

VEGETATIVE GROWTH RATE (WEIGHT) OF *P. palmata* GROWN IN THE ONSHORE TANKS.

TANK NUMBER: RECTANGULAR TANK 2

DATE	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	NO OF DAYS	R^W	HOURS OF SUNSHINE
29/6/87 - 5/7/87	850	980	7	0.0203	51.5
5/7/87 - 12/7/87	850	1025	7	0.0267	61.4
12/7/87 - 19/7/87	850	990	7	0.0218	18.4
19/7/87 - 26/7/87	850	992	7	0.0221	66.2
26/7/87 - 2/8/87	850	969	7	0.0187	19.5
2/8/87 - 9/8/87	850	988	7	0.0215	53.7
9/8/87 - 17/8/87	850	1003	8	0.0267	38.2
17/8/87 - 23/8/87	850	953	6	0.0191	19.8
23/8/87 - 30/8/87	850	951	7	0.0160	39.0
30/8/87 - 6/9/87	850	996	7	0.0228	46.0
6/9/87 - 13/9/87	850	980	7	0.0203	33.5
13/9/87 - 20/9/87	850	994	7	0.0224	47.0
20/9/87 - 27/9/87	850	959	7	0.0172	41.5
27/9/87 - 4/10/87	850	970	7	0.0189	36.5
4/10/87 - 11/10/87	850	964	7	0.0180	18.8
11/10/87 - 18/10/87	850	960	7	0.0174	37.6
18/10/87 - 25/10/87	850	995	7	0.0225	21.1

APPENDIX 4

Final and initial length (mm) from different parts of *Palmaria* thalli data.

Sample	Tip		Middle		Base		Total Length		Time
	F	I	F	I	F	I	F	I	(Day)
11	46.08	14.62	28.35	20.69	20.30	18.02	94.73	53.33	22
9	52.40	24.12	23.22	20.60	29.70	28.15	105.32	72.87	14
7	46.50	14.57	**30.70**	**21.50**	**17.85**	**16.25**	95.05	51.34	22
5	55.77	28.34	32.07	30.76	43.53	40.18	131.37	99.28	14
2	53.40	13.80	13.80	29.20	19.97	29.23	24.00	111.83	22
23	36.37	13.33	26.55	19.85	6.12	5.78	69.04	36.96	22
18	42.50	15.60	24.54	20.61	19.77	18.93	87.21	55.14	22
26	49.28	29.55	24.38	20.23	34.00	28.65	107.66	78.43	22
21	46.98	16.15	28.54	20.29	16.23	15.30	91.75	51.74	22
4	49.12	24.89	**21.45**	**20.50**	**28.60**	**26.24**	99.17	71.63	14
20	31.02	14.00	23.98	20.15	18.25	17.17	73.25	51.32	14
30	38.60	16.22	27.88	19.70	20.50	18.40	86.48	54.32	22
28	30.80	14.72	22.65	20.05	20.85	19.30	74.30	54.07	22
27	43.60	16.30	25.95	19.92	21.65	20.50	91.20	56.72	22
25	45.47	26.75	21.20	20.50	30.10	29.17	99.77	76.42	14
6	52.05	16.10	28.65	18.60	26.00	24.15	106.70	58.85	22
12	76.12	33.55	23.60	20.54	29.30	27.77	129.02	81.86	22
3	71.47	26.45	36.70	30.70	32.35	29.30	140.52	86.45	22
16	41.00	13.35	31.10	19.63	29.80	26.90	101.90	59.88	22
19	52.90	25.57	25.05	20.55	21.92	20.50	99.87	66.62	14
24	50.33	35.05	**20.75**	**20.05**	**40.40**	**39.80**	112.48	94.90	14
15	38.77	26.70	**22.00**	**19.87**	**36.75**	**33.00**	97.52	79.57	22
77	63.20	39.97	35.35	30.23	51.50	48.80	150.05	119.00	13
29	52.63	28.41	24.71	20.94	29.25	17.78	106.59	76.88	14
66	81.43	61.50	32.10	31.70	50.20	47.50	163.73	140.80	13
17	59.57	25.15	26.50	20.67	25.13	22.60	111.20	68.42	22
12	32.05	16.00	21.40	19.80	15.00	14.90	68.45	50.70	14

F= Final length I= Initial length

Number in bold shows where R^L at the base were higher than at the middle.

APPENDIX 5

Final and initial area (mm^2) from different parts of ***Palmaria*** thalli

data.

Sample	Tip		Middle		Base		Total Length		Time
	F	I	F	I	F	I	F	I	(Day)
11	1410.86	186.07	625.95	313.57	209.72	168.68	2246.53	668.32	22
9	1605.73	395.48	487.23	347.57	397.57	347.77	2490.53	1090.82	14
7	833.89	127.47	603.92	257.05	162.48	139.21	1600.29	523.73	22
5	935.52	301.24	568.70	477.15	511.36	436.83	2015.58	1215.22	14
2	1927.63	214.50	628.57	304.03	273.39	193.09	2829.77	711.62	22
23	523.28	88.22	391.97	200.72	39.39	30.50	954.63	319.44	22
18	600.35	108.46	700.08	242.99	144.95	125.64	1445.38	477.09	22
26	682.35	300.42	346.58	235.05	368.23	261.33	1397.16	796.80	22
21	809.28	111.96	499.07	227.61	89.59	74.57	1397.93	414.13	22
4	1195.06	334.16	**364.72**	**329.73**	**273.99**	**237.03**	1833.77	900.92	14
20	390.70	98.80	307.96	223.10	137.39	121.04	836.05	442.93	14
30	977.73	226.85	574.01	279.52	223.73	165.82	1785.47	672.19	22
28	874.19	194.70	371.53	285.86	201.72	163.22	1447.44	643.78	22
27	1021.73	175.97	783.40	301.04	188.80	167.92	1993.92	644.92	22
25	1046.97	421.45	**391.02**	**379.72**	**373.15**	**343.51**	1811.14	1144.67	14
6	1162.57	148.83	584.22	286.51	329.76	265.09	2076.55	699.93	22
13	2296.98	486.60	424.55	318.02	306.47	287.40	3028.00	1092.02	22
3	2124.68	383.07	856.99	577.75	346.47	297.94	3328.14	1258.76	22
16	822.29	123.93	656.23	282.33	329.96	233.53	1808.48	639.79	22
19	1216.97	364.90	578.39	351.53	242.33	210.64	2037.69	927.07	14
24	1362.78	768.37	**519.31**	**495.42**	**587.60**	**510.99**	2469.68	1774.77	14
15	1146.88	647.40	735.31	551.40	587.10	484.67	2469.28	1678.45	22
77	2125.41	1061.16	1331.51	1103.40	960.30	847.46	4417.21	3012.02	13
29	1158.93	454.29	559.72	373.45	370.18	320.43	2088.83	1148.17	14
66	3198.36	2177.18	952.14	910.95	793.26	774.45	4943.76	3862.57	13
17	2048.62	492.37	638.57	349.06	244.39	189.88	2931.58	1031.31	22
12	500.40	165.14	287.72	261.09	108.21	102.94	896.33	529.17	14

F= Final area I= Initial area

Number in bold referred to area at the base which were higher than area

at the middle.

APPENDIX 6

Final and initial length (mm) of partially damaged and normal tips data

Partially damaged tip			Normal tip			
Sample	Final	Initial	Sample	Final	Initial	Time (Day)
1	86.35	83.55	1	105.00	98.20	22
2	71.65	61.50	2	95.45	84.35	22
3	81.75	70.85	3	104.05	100.45	22
4	87.85	82.55	4	97.80	86.20	22
5	131.50	127.90	5	85.45	77.25	22
6	75.85	71.70	6	63.20	54.00	22
8	57.45	54.20	7	79.90	65.00	22
9	81.35	73.40	8	96.55	86.10	22
10	61.55	59.80	9	76.35	65.60	22
11	85.20	79.50	10	121.25	109.55	22
12	77.70	71.05	11	95.05	82.25	22
15	68.10	65.70	12	105.85	100.25	22
16	78.00	75.10	13	114.60	105.45	22
17	67.30	60.45	14	86.10	73.70	22
18	65.60	59.95	15	125.05	112.60	22
19	93.30	79.70	16	98.10	87.95	22
20	63.35	57.50	17	95.45	87.35	22
21	67.65	63.80	18	90.20	76.65	22
22	59.00	57.15	19	102.05	94.55	22
23	90.30	83.75	20	114.35	102.80	22
25	76.00	70.40	21	84.65	78.60	22
26	55.80	54.15	23	114.40	105.15	22
27	69.00	60.75	24	97.30	91.05	22
28	91.65	81.80	25	101.75	95.35	22
29	62.80	55.45	26	84.80	75.80	22
30	61.10	55.25	28	810.75	71.40	22
*	*	*	29	98.95	88.85	22
*	*	*	30	91.70	86.20	22

AN ASSESSMENT OF POTENTIAL OF THE RED SEAWEED *PALMARI PALMATA* FOR MARICULTURE IN THE IRISH SEA

ABSTRACT

The aim of this study was to grow ***Palmaria palmata*** as a potential food source. In the laboratory methods of obtaining spores, optimum conditions for spore germination and growth of sporelings under various photon irradiances, different photoperiods and in different growth media were investigated. Growth pattern of ***Palmaria*** plants were also studied under laboratory conditions. Three methods of growing ***Palmaria*** were investigated: growing vegetatively in onshore tanks using continuous flow and batch systems; seeding cords with spores and inserting fragments into cords and transplanting them to the sea.

The viability and percentage germination of spore fluctuated. Gentle filtration and addition of plant growth hormone, indole-3-acetic acid improved germination. Photon irradiance between 16 and 30 umolm^{-2}s^{-1} appeared to give best germination of about 40%. ***Palmaria*** sporelings seemed to grow best in Provasoli's ES media half strength in filtered seawater and 16:8 h (light:dark) photoperiod.

The growth pattern study showed that growth rate in length (R^L) at the tip was about twice that of the whole thallus. R^L gradually decreased from the tip to the base. R^L in width was faster at the middle. Plants with partially damaged tips showed a significant reduction in growth rate in length but not in area and weight. Plants with severely damaged tips showed a significant reduction in growth rate in terms of weight. There was a clear relationship between relative growth rate in term of length, area and weight. Area growth rate was about twice and weight growth rate was about 2.8 times the length growth rate.

Growing ***Palmaria*** vegetatively in onshore culture tanks was not economically viable in Port Erin, Isle of Man because of the high cost of pumping seawater, collecting ***Palmaria*** plants and labour for maintaining such systems. The inability of ***Palmaria*** plants to produce marginal proliferations at some time of year and the need to replace the plants periodically perhaps is one factor why ***Palmaria*** is not suitable for tank culture.

Growing ***Palmaria*** from spores and fragments on cords in the sea suffered from one common problem, fouling by unwanted seaweeds, particularly Laminariales. Growing ***Palmaria*** from spores had many problems such as low percentage germination,inability to form secure attachment on cords and inability to compete with fouling diatoms and Laminariales. Growing from fragments gave more encouraging results. Thalli produced marginal proliferations and developed into plants with sizes ranging from less than 2 to 129g.