Effects of Sediments on the Development of *Macrocystis pyrifera* Gametophytes

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Abstract

Rocky ocean bottoms are covered from time to time with small amounts of fine sediments. This material may interfere with the development of germling stages of *Macrocystis pyrifera*. Tests were performed by introducing sediments before and after spores were dispersed in culture dishes. 10 mg cm^{-2} of sediment, enough to occlude the surface, prevents spore attachment, greatly reducing the probability of survival. Smothering of established germlings was severe at 108 mg sediment cm^{-2}. Water motion further reduced spore success where sediments were present, probably because of abrasive scour. Mechanisms of natural and human interference with *M. pyrifera* reproduction are suggested.

Introduction

Beds of the giant kelp *Macrocystis pyrifera* are a valuable ecological and economic resource in southern California, USA. Reduction in the extent of the beds, which began in the 1950's (North, 1967) has thus caused concern. Because the earliest and most severe declines were seen near centers of population, it has been presumed that pollutant discharges or other human activity were contributory. However, the immediate causes are complex. No direct toxic effect has been generally demonstrated for sewage treatment plant effluents.

Macrocystis pyrifera is a favored food of sea urchins (Leighton, 1966). North (1974) has accumulated considerable evidence suggesting that the kelp bed decline is attributable to the loss of natural controls on urchin populations. A major predator of adult urchins, the sea otter, has been eliminated in southern California. Because pathways developed by which urchins could derive nourishment from sewage discharges, starvation was made less effective as a population-limiting factor in outfall areas. Thus, urchins became dominant, destroyed the kelp beds, and remained indefinitely.

It is also possible that growth in kelp beds has been reduced by increased water turbidities. Light levels are often a limiting factor, particularly in the success of juveniles (Clendenning, 1964; Rosenthal *et al.*, 1974). Sewage disposal or dredging activities may have reduced water clarity and thus photosynthesis.

The decline of any particular bed probably resulted from some combination of these factors and occasional adverse natural conditions. Considerable damage has been associated with unusually warm waters or heavy storm surge.

North (1970) has suggested another factor which may reduce kelp bed success. Fine sediments, settling from time to time on rock surfaces, may interfere with gametophyte development.

Ecological effects of sediments on rocky-bottom organisms have been widely recognized in past studies. Lilly *et al.* (1953) described effects of sediments on faunal distributions in a small bay in Ireland and suggested several immediate mechanisms, including prevention of larval attachment, smothering of larvae, reduction of oxygen concentrations, production of carbon dioxide or hydrogen sulfide, and effects through competitors or predators. Ebling *et al.* (1960) suggested that the distribution of *Laminaria* sp. in the same area was limited by sediments, and that sediment removal by turbulent water favored the plants. Neushal *et al.* (1976) studied recruitment on artificial substrate in southern California waters, and concluded that *Macrocystis* sp. success

was reduced where sediment layers were present.

Sediments affecting kelp beds could be of natural or human origin. Periods of calm weather and turbid water may produce sediment layers. But man adds sediment to ocean waters in a variety of ways: sewage disposal, dredged material disposal, enhancement of erosion, etc. While the amount of sediment present on rocks as a result of man's activities has never been generally measured, extreme examples exist. We have seen layers up to 1 cm thick on breakwater rocks 30 ft (10 m) deep inside a harbor, where turbulence is reduced and artificial and natural sediment sources are present. It is probable that many other areas are affected to lesser degrees.

At least 4 mechanisms of sediment damage to germlings may be postulated: (i) If the material is on the rock surface when the spore settles, attachment to the rock may be prevented; attachment to a sediment particle would result in eventual loss of the plant to wave turbulence. (ii) Once the spore has settled and attached to clean rock, an ensuing episode of sedimentation could smother it, cutting off light or nutrients. (iii) If sediment is present where waters are turbulent, spores and gametophytes could be damaged by scouring action. (iv) Sediments could change the chemical nature of the microenvironment surrounding the spore; interstitial concentrations of toxic pollutants might be high, or oxygen could be depleted; bacteria or other predators might be encouraged.

The experiments reported here were designed to investigate the first three of these mechanisms.

Materials and Methods

Culturing

Sporophylls were obtained from a *Macrocystis pyrifera* bed on the Palos Verdes peninsula in southern California. They were wrapped in damp cloths and kept cool during transport to the laboratory. The blades were wiped to remove mucus (which encourages bacterial growth), and totally immersed in a beaker of filtered seawater at 8° to 10°C. Spores were released in 30 to 45 min, and 1 ml of the inoculum was transferred to petri dishes. The dishes contained microscope slides and either 19 or 29 ml of seawater. The water was obtained from Kerckhoff Marine Laboratory at Newport Bay, and had been sand-filtered. Water from the bay is usually nutrient-rich. In each case, the inoculum was examined to determine that active spores were abundant. Germinating spores in control dishes were counted to determine the health and density of each set of cultures. Densities in control cultures ranged from 150 mm^{-2} to as high as 8000 mm^{-2}. North (1969) has grown plants successfully at such densities, and no effects of density were seen in these experiments. The culture dishes were maintained in a continuously lighted refrigerator at 8°C.

Counting

The success of the experimental cultures was measured by counting the number of attached, normally developing germlings. Mean counts in experimental dishes were divided by those for control plates to determine "relative survival".

During normal development the spores (about 5 μm diameter) develop germ tubes within a few hours to a few days. The tube is extended, and after a day or two more, the gametophyte develops at the distal end. During the subsequent weeks the gametophytes continue to grow, and usually develop plastids.

In early experiments, counts of normally developing germlings were made at 4, 8, and 16 days. No significant differences between subsequent counts for the same culture dish were found, however. Apparently any harmful effects of the sediment occur immediately. Therefore, the remaining experiments were run for only 4 days, considerably speeding up the procedure. Spores were counted as surviving if germination had occurred.

For each count, the slide was removed from the petri dish, rinsed gently to remove sediment, and protected with a cover slip. From a haphazardly chosen starting point, a grid of 9 microscope fields was examined, and the number of germlings within each counted. The mean of the 9 counts is reported herein.

Sediments

The test sediment used was construction-sand sieved through number 200 mesh, which passes particles of 74 μm diameter or smaller. Volatile solids content, determined by heating to 550°C for 20 min, was 3%. This material was chosen on the presumption that it would be chemically and biologically inert.

The sediment was spread in the petri dishes by mixing it vigorously with a measured amount of water and pouring the mixture into the plate. Care was taken to produce a uniform layer. The

amounts of sediment used are reported as mg cm^{-2} (dry weight). Amounts of sediment greater than about 10 mg cm^{-2} formed layers which completely occluded the slide surface. At less than 10 mg cm^{-2}, the sediment "layer" consisted of isolated particles or groups of particles separated by clear substrate. During experiments for which dishes were agitated, the sediment formed uneven, shifting deposits.

Interference with Attachment

The effects of sediment on the spore attachment process were first tested by introducing inoculum with a pipet to petri dishes with sediments in place. Because this sometimes disturbed the sediments, and may not have provided a random dispersal of the spores, subsequent experiments were performed by mixing spores and sediment together. Because the sediments settled more quickly than the spores, this procedure still represented a test of spore penetration of an existing layer. Results were comparable for both procedures.

Spore Burial

In the second series of experiments, spores were allowed 24 h to attach to the slide before sediment was added. The culture was initiated in 20 ml of seawater, and sediments suspended in 10 ml, so that the dish finally held 30 ml, as in the other experiments.

Water Motion

Effects in moving waters were tested by mounting the experimental cultures on a shaker (Derrick Manufacturing Company, Model 150). An oscillatory motion with an amplitude of 5 mm and a frequency of 5 cycles per second was produced. Average water velocity was thus approximately 5 cm sec^{-1}.

Analysis

In each experimental run, mean spore density in the control plates was used as standard. Mean densities on the experimental plates were divided by the standard to produce relative survival rates, expressed in percent. Several functional relationships between relative survivals and the amounts of sediment present were examined. In each case, a declining exponential gave the best

least-squares fit to the data. Results are expressed in the form,

$$S = Ke^{-kq},$$

where S is relative survival in percent, q is the quantity of sediment present in mg cm^{-2}, and K and k are constants calculated in the least-squares approximation.

Results

Interference with Attachment

When sediment was introduced prior to dispersal of *Macrocystis pyrifera* spores, or when spores and sediment were introduced simultaneously, even small amounts of sediment sharply reduced survival rates (Fig. 1). The least-squares relationship was,

$$S = 34e^{-0.29q},$$

8 mg cm^{-2} of sediment reduced survival by about 90%. Microscopic examination of the slides suggested that 10 mg cm^{-2} of sediment was the amount which produced
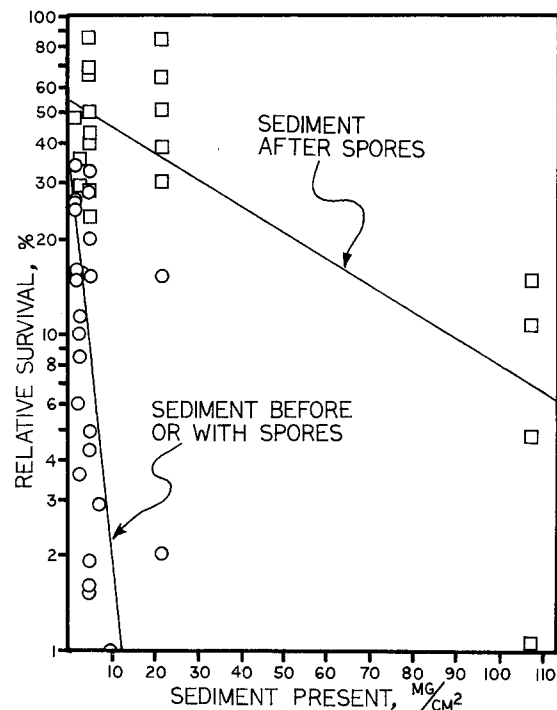

Fig. 1. *Macrocystis pyrifera*. Relative survival of spores versus amount of sediment present. Circles: experiments in which sediments were applied before spores or were initially mixed with spore solution; squares: experiments in which spores were given 1 day to attach before sediment was added. One point (sediment present = 9.4 mg cm^{-2}, relative survival = 0.5%) is not shown

essentially complete coverage of the slide. Estimates were made by eye of the fraction of substrate left uncovered by amounts less than 10 mg cm^{-2}. Within the accuracy of the estimates, relative survival was equal to the uncovered area of the slide expressed as percent of the total surface.

Spore Burial

Spores given an initial 24 h to attach before sediment addition were considerably more resistant to damage (Fig. 1). Survival was relatively high under 20 mg cm^{-2} of sediment, and about 10% of the spores developed even under 108 mg cm^{-2} (a layer about 450 µm thick). The least-squares relationship was,

$$S = 54e^{-0.019q}.$$

An additional experiment was performed to determine whether failure of spores covered by sediment could be attributed to loss of light. Two sets of spore cultures were covered with various amounts of sediment 24 h after dispersal. One set was placed on an opaque tray, so that light from above, which could be blocked by sediment, was the only significant source. The second set was placed on a transparent tray, so that light from below could reach the spores. The cultures were examined after 4 days. Under both lighting conditions, the effects of various amounts of sediment were similar to those found in previous burial experiments. Thus, sediment effects were not attributable to light deprivation.

Water Motion

Experiments were performed to determine the effects of moving water in two ways. In the first, the sediments and spores were mixed and allowed to settle for 1 day before agitation began. In the second, agitation began immediately. Growth was compared to results from the "interference with attachment" experiments which used sediment-spore mixtures (Fig. 2). Least-squares relationships found were:

for no motion: $S = 32e^{-0.31q}$
for motion after 1 day: $S = 33e^{-0.43q}$
for continuous motion: $S = 40e^{-0.59q}$

When no sediment was applied, the mean survivals were lower in moving waters (although the difference was not statistically significant).

Addition of sediment reduced survival in both still and moving waters. The ef-
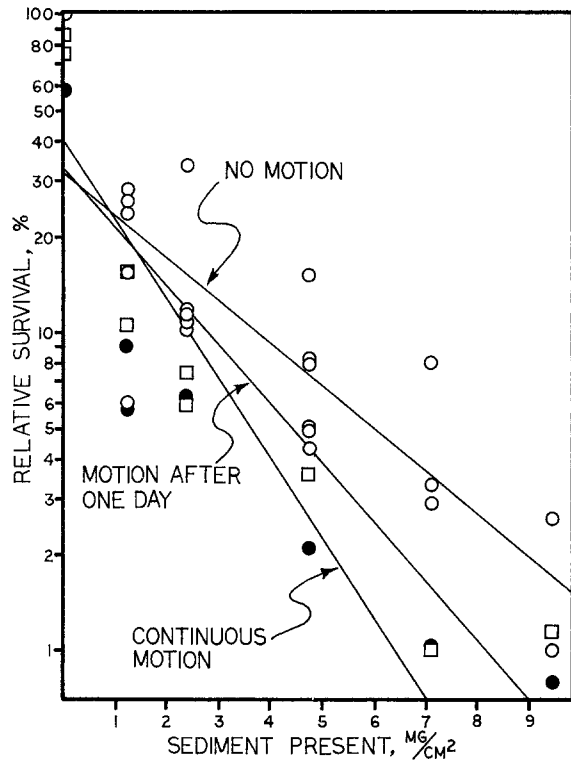

Fig. 2. *Macrocystis pyrifera*. Relative survival of spores versus amount of sediment present for different water motion regimes. Open circles: no motion; squares: motion after 1 day; filled circles: continuous motion

fect was more severe in moving water, however. At 7.1 mg cm^{-2}, the difference between still and agitated plates is highly statistically significant. Statistical significance was lost in this experiment at 9.6 mg cm^{-2}, but it is likely that this is an artifact: surviving gametophytes were so few in all plates that sufficient counts could not be made.

Allowing a day without agitation for spore attachment to occur did not significantly alleviate motion damage.

Discussion

Interference with *Macrocystis pyrifera* spore attachment by rock-surface sediment may be explained by at least two possible models. Either the spores recognized the sand grains as unsuitable, and delayed settlement, or they attached to the grains and were lost when the slide was washed. While our results were not conclusive, we believe they favor the second explanation. At least some propagules capable of choosing substrate become progressively less discriminating with time — but the 16-day experiments with the *M. pyrifera* spores showed no

greater attachment success than the 4-day experiments. Secondly, for amounts of sediment less than 8 mg cm^{-2}, bare substrate was available. Thus, if the spores were actively searching for firm surface, they all would have attached between the grains. But within the accuracy of our simple estimates, relative survival was equal to the fraction of substrate left unoccluded. The results are consistent with the hypothesis that the spores attached to whatever they first encountered, and that the probability of encountering a sand grain was roughly equal to the surface area covered by the sand. Because spores attached to sand grains are unlikely to survive in the wild, we believe that losses measured in the laboratory are a meaningful indication of losses under natural conditions. Attachment to particles has been demonstrated by others (North, 1970).

The effects of burial have also been previously observed in culture. North (1970) suspended a slide seeded with embryo sporophytes in Newport Bay. When portions of the slide were covered with sediment, sporophytes in those portions did not develop. Adjacent, uncovered areas of the slide supported normal growth.

Because light deprivation was shown to be unimportant, and predators and competitors were excluded from the cultures, the effects of burial probably arise from alteration of the chemical or physical characteristics of the mircoenvironment of the germling. Further experiments using sediments of various compositions would be appropriate.

The effects of water motion on spore attachment were detrimental. Such effects without sediment have been previously documented. North (1969) reported experiments in which moving slides were exposed to inoculum for 4 min. Rates of movement comparable to those described here significantly reduced spore attachment success. Charters et al. (1973) tested the effects of moving water without sediment on spores after their attachment. Swiftly moving water did dislodge some germlings. The resistance of the germlings changed with time after attachment, rising for some species and falling for others.

The experiments reported here were consistent with these previous results. Water motion reduced relative survival, and continuous motion was more harmful than motion after 1 day (although not all comparisons were statistically significant).

Where sediments were present, survival was further reduced. Thus, water mo-

tion and sediments were both detrimental, and the effects in combination were more harmful than either alone.

Ecological effects of sediment scour have been suggested. Kitching et al. (1934) hypothesized its importance to algae distributions in a submarine gulley in England. However, in natural systems effects may be more complex. *Macrocystis pyrifera* grows well in turbulent waters. In cultures in partially filtered water, we have found severe turbulence aids *M. pyrifera* germlings by washing away competing diatoms.

The small amount of sediment sufficient to reduce germling survival suggests such phenomena may be significant in the sea. Layers of fine sediments are commonly noted on rocky substrate by divers, particularly in areas of relatively still water. The nature of such sediments is highly variable. They may be clay, sand, or organic material, may occur with various particle size distributions, and may or may not include pollutants. The fine sand used for these experiments is only one example of the many possible materials. It was chosen to provide a simple starting point for the investigation. Full understanding will require work with many other sediments. For some, particularly those including pollutants, chemical and secondary biological effects may be important.

Conditions which allow sediment layer formation may be a natural disaster for algae reproduction. Lilly et al. (1953) found 15 to 40 mg cm^{-2} of sediments in sheltered areas in their study area, and suggested they may cause the absence of some animals from bays and particularly from horizontal surfaces such as the tops of rocks. Kitching et al. (1934), Kitching (1941) and Norton et al. (1977) observed algae distributions which they felt were controlled by sediments. Dahl (1971) detailed the remarkable resistance of *Zonaria farlowii* to complete burial, showing extreme adaptation to what must be an important ecological factor. Foster (1975a) suggested that occasional burial by shifting sand may be an important factor in maintaining subtidal diversity because it clears substrate for eventual recolonization. Specifically concerning *Macrocystis* sp., Foster (1975b) and Neushal et al. (1976) found the young plants to be more successful near the edges of plates of artificial substrate and on wire mesh, where sediments were less prevalent.

While there is abundant evidence that sediments on rock surfaces are important, the phenomenon is also complex. Norton et al. (1977) noted that sediments present, and their effects, varied with sub-

strate slope, water depth and turbulence. Kitching *et al.* (1934) and Kitching (1941) mentioned substrate slope and wave action. The work of Neushal *et al.* (1976) suggests the importance of the small-scale shape of the substrate.

All of the investigators pointed out the importance of temporal variation. Neushal *et al.* in particular measured sedimentation rates and noted seasonal variation: more material settled from the water in winter. Removal of sediments from a plate at intervals (25 times in a year) had no biological effect, presumably because the sediments were rapidly replenished by natural sources.

Damages resulting from human interventions are possible. Sewage disposal, dredging, ocean dumping, current changes caused by breakwaters or power plants, and other activities may change the thickness of sediment layers or the length of time they are present. It is possible that these factors have contributed to kelp bed decline in some areas. Adequate evaluation of this possibility awaits an extensive survey of rock-surface sediments. It would be valuable to know the dynamics of sediment layers in various polluted and unpolluted environments.

It may be desirable to adjust kelp bed restoration efforts to allow for sediment effects. Certainly areas where rocks are commonly covered should not be chosen as transplant sites. If seasonal variation in the presence of sediment can be found, transplant schedules might be adjusted accordingly.

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