SEDIMENT AS A CAUSE OF MACROCYSTIS GAMETOPHYTE NON-SURVIVAL

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Abstract
Survival of Macrocystis spp. gametophytes is a function of sediment depth on the surface to which they attach. Relative survival varies as an exponential function of calculated mean depth in all cases. If spores attach to a surface prior to sediment settlement, no survival is noted after four days at depths above 500 microns. If spore inoculation occurs after settlement, no survival is noted for calculated mean depths above 40 microns. Microscopic examination of silt dispersal patterns showed that below 40 micron depths the percentage of non-occluded surface was also an inverse exponential function of mean sediment depth. We infer that motile zoospores cannot pass through even the finest silt particles. Comparatively better survival is noted with attachment prior to deposition.

1. Introduction
Considerable concern over the disappearance and fluctuation in density of giant kelp (Macrocystis spp.) beds along the southern California coastline has been shown recently. Fisherspersons (sic), sports divers, environmentalists and others value this algae as a vital part of the complex marine ecosystem. Commercially, the giant kelp plant is a principal source of alggin, a highly efficient thickening and gelling agent. Kelp is also a good source of potash and other useful chemicals (Limbaugh, 1955). More recently, concerns over limitations of food and energy resources have pointed towards kelp as a promising panacea. The former Energy Research and Development Administration's Ocean Farm Project is an example of one of these later schemes.

Giant kelp beds apparently have diminished both in size and density as the human population of the coast of west America has grown. Although evidence is confusing and difficult to evaluate, North (1970-74) and others contend that Macrocystis tends to flourish along coastlines with less dense populations, compared with it's growth off heavily populated regions such as the Los Angeles basin.

Many hypotheses have been formulated to explain the alleged kelp bed diminishment. They include sea urchin proliferation at sewage outfalls (North, 1970-74), near-extinction of the sea otter (Kesten, 1974), increased water turbidity near sewage outfalls (Neushal, 1976), presence of toxic chemicals or pesticides (Barilotti, 1976), and sediment settlement on rock substrate (Devinn, 1977).

The purpose of this paper is to outline certain experiments conducted to test the latter hypothesis that sediment deposits (whatever their origins) might be a direct cause of Macrocystis spp non-survival (Volse, 1977). The studies described herein deal only with the effects of varying the sediment depths under laboratory conditions. Timing of spore inoculations (i.e., whether spores were inoculated prior to or after sediment settlement) proved to be a most important parameter in all of the experiments conducted.

2. Experimental Methods
Procedures for Macrocystis spp. laboratory culture have been developed by Neushal (1963), North (1970) and others. By modifying existing techniques, a standard procedure was developed by the author to provide for statistical analysis throughout the course of his experiments. Zoospores from Macrocystis spp. sporophylls, through chilled seawater temperature shock, were released to form a spore solution. These spores were incubated under controlled conditions of temperature and lighting for a standard period of four days. Various combinations of sediment with and without spore solution went into an array of petri dishes, each containing a microscope slide in conjunction with an appropriate amount of nutrient-rich seawater. Temperature was kept at 80°C throughout all experiments. Four days after inoculation of zoospore solution, slides were removed, sediment washed clean, and a slide cover put in place. Under a light microscope of appropriate power to insure statistical significance, counts of surviving gametophytes were made. A typical appearance of these germlings after four days incubation is shown in the microphotograph of figure 1.
To measure a particular inoculation of spores' ability to attach to substrate and survive conditions imposed experimentally, a measure, called gametophyte survival index (GSI) was devised. One petri dish of each experimental array was designated as a "control" dish, with no sediment to be introduced therein. Four days following inoculations, germling counts were made on both control and experimental slides. GSI is defined as the ratio of a field count for an experimental sedimentary slide to a field count for the control slide without sediment. Setting control slide GSI at 100, GSI on a scale from 0 through 100 becomes a relative measure of gametophyte survival under each of the imposed environmental conditions.

Amounts of sediment calculated to settle compactly at its specific gravity were weighed into plastic bottles along with volumetric measurement of sea water media (enough for duplicate samples). In cases where sediment was added concurrently with spore inoculation (providing opportunity for sediment to settle prior to spore attachment), each dish received 30-ml of well-shaken media-spore-sediment suspension. In cases where sediment was added 24 h later, only 20 ml of media-spore solution first went into dish. The following day an additional 10 ml suspension of media and sediment followed.

Source of Macrocystis spp. zoospores was from sporophylls obtained from the base of plant growing in the vicinity. When immersed in chilled seawater media at 80°C, temperature shock released spores giving a golden-brown color to the solution. A one-ml amount of spore solution went into the sediment-media bottles, and after careful shaking, inoculation into petri dishes was then carried out volumetrically.

To guard against mistakes and to provide a better statistical basis, each of the experiments was run in duplicate. Geraldings were counted in the field of either a 500x or 125x light microscope, depending upon the quantities to be visually counted. Nine random counts were made on each experimental slide. Data was compiled and is shown in Tables 1, 2, and 3. Regression analysis is the basis of all statistical testing. Empirical equations fit data selected by the method of least squares. Curves of the type $y = a + bx$, $y = e^{mx}$, and $y = mx^n$ were investigated and the type selected which showed the best correlation coefficient, even when significant fits with lesser correlation might be found.

3. Gametophyte Survival as a Function of Sediment Depth

The totality of experiments established a relationship between GSI and mean calculated sediment depth. Best fit for all data proved to be given by an equation of exponential type, plotting on semi-logarithmic paper as a negatively sloped straight line. Using statistical tables based on quantity of data and correlation coefficients obtained, indicates 99+ percent probability of such data relationship being valid.

Consolidated data from Tables 1 and 2 gave the best equation for the case where spore solution was introduced simultaneously with or prior to sediment placement as

$$GSI = 34.3 e^{-0.088d}$$

Eq. 1

where GSI is in percent and sediment depth, $d$, is in microns. For 37 data pairs, correlation coefficient is -0.777.

In cases where spores were allowed to attach to slide prior to sediment introduction, the empirical equation is of the same type as Eq. 1 but the exponential coefficient is 1/200th that of Eq. 1 With data from 23 observations (Table 3), best equation is

$$GSI = 54.4 e^{-0.0044d}$$

Eq. 2

with a correlation coefficient of 0.792. Plot of these data along with corresponding empirical equations are shown in Figure 2.

4. Partial Slide Occulsion by Thin Sediment Layers

An examination of several slides before sediment had been washed off prior to germling counts revealed that, owing to gradation of particle sizes (i.e. according to Figure 3, less than 10 percent are smaller than 10 micron diameter) slides with calculated mean depths of up to 20 microns were not fully occluded by sediment. Table 4 indicates the observed relationship between calculated mean sediment depth and the observed percentage of field which appeared as sediment-free. A plot of these data is given in Figure 3, for which the empirical equation

$$PSF = 103 e^{-0.127d}$$

Eq. 3

was obtained. PSF is the observed percentage of field that was sediment-free and d is the calculated mean sediment depth (up to about 40 microns). With data from 24 observations during one experiment, a regression coefficient of $r = -0.966$. This is far greater than $r_{(crit. 1%)} = -0.462$, showing an extremely high statistical significance. At mean calculated sediment depth of 40 microns or greater,
sediment coverage proved to be complete in all cases viewed. The prime factor involved here seems to be particle occlusion of a surface. Long (1976) remarked that germlings have been observed to attach themselves to small particles and continue their growth under laboratory conditions. Such attachment, although possible, would seem to provide an uncertain fate for the gametophyte, being subject to whatever water currents nature provided. It seems apparent that such attachment to fine particles would prove futile insofar as ultimate sporophyte survival is concerned.

Figure 2. Effects of sediment on survival of Macrocystis pyrifera gametophytes, including effect of timing of spore inoculations. Data taken from Tables 1-3.

Figure 3. Sediment grain size based on hydrometer analysis of sediments. All particles are smaller than 74 micron diameter.

We note from Equation 3 that the percentage of field sediment-free (PSF) is an inverse exponential function of sediment depth, d, as is the gametophyte survival index (GSI) of Equation 1. While the equations are not identical, comparisons over much of the range examined (Figure 3) are consistent with a simple explanation: the spore produces a germling attached to the slide only if it falls on exposed glass. If the spore falls on sediment particles, it either fails to develop or is lost when the slide is rinsed. Because a spore attached to sediment is unlikely to survive under natural conditions, experimental survival rates probably are similar to those under appropriate circumstances in the ocean.

5. Summary and Conclusions

Macrocystis pyrifera gametophyte survival is inversely related to sediment depth. In cases of sediment introduction either before or along with zoospore inoculation, the best empirical equation
corresponding to 37 data pairs (Fig. 2) is

\[ GSI = 34.3 e^{-0.088d} \]  

where \( GSI \) is gametophyte survival index in percent and \( d \) is mean sediment depth in microns. In cases where spore inoculation occurred 24 hours prior to sediment placement, the best empirical equation corresponding to 23 data pairs (Fig. 2) is

\[ GSI = 54.4 e^{-0.0044d} \]  

Excellent statistical correlation exists for both equations showing the relationship between variables to be highly significant.

Examination of slides made prior to counting procedures revealed that field coverage by particles is variable, increasing with sediment depth. According to a plot of twenty data pairs (Fig. 4), the best empirical equation is

\[ PSF = 103 e^{-0.127d} \]  

where \( PSF \) is percentage of surface sediment-free and \( d \) is sediment depth in microns. We can conclude that although Equation 3 is not identical with Equation 1, comparison over most of the range examined is consistent with the explanation that a spore produces a germling attached to a slide only if it falls on exposed glass.

By comparison of Equation 1 and Equation 3, and by rounding off the exponential exponent to 0.1 in both cases, we can conclude that an approximate linear relationship exists between GSI and PSF, up to a calculated sediment depth of some 40 microns. It appears that the diameter of particles at these low depths does not control the relationship, but rather the degree of distribution of these particles in occluding the slide. When sediment is in place prior to zoospore arrival, presence of any sized particle is sufficient to impede attachment of zoospore to substrate. Even though spores may manage to attach themselves to coarse sediment particles, the ultimate instability of the particle seems to preclude gametophyte survival.

Whenever spores attach themselves to a surface prior to sediment introduction, gametophyte survival chances are greatly increased. We infer that the effect of sediment in this case lies not in interfering with the germling's continued attachment, but rather in preventing nutrients or oxygen from being absorbed by the attached gametophyte.
References


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