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An analysis of effect of diatoms
on growth of *Gigartina stellata* sporlings

Introduction

Gigartina stellata is a macroalgae and a member of the red algae (Rhodophyta) of the family Gigartisaceae. They commonly inhabit the temperate or tropical lower shore marine waters, rocky shores, and rock pools and occasionally can be found growing under large brown seaweeds (fucoids) on less exposed shores. Commonly known as False Irish Moss and often confused with *Chondrus crispus* (Irish moss) *G.stellata* is a source of carrageen which is used to make soups and jellies as well as a remedy for respiration disorders in Ireland and is economically very important as a source of agar and agarose (Buschmann *et al*, 2001). Recently *G.stellata* has been renamed and given the name *Mastocarpus stellatus*. For the duration of this report it will be referred to as *G.stellata* as this fits with references to previous literature that is being referred to. Sporlings of *G.stellata* are disc-like and need to attach to a substrate to grow effectively. This could be anything from a rock surface to a mollusc shell. Because of *G.stellata* economic as well as natural importance research into factors affecting the attachment and subsequent growth of *G.stellata* (inhibiting factors) for example the effects of diatoms is important.

Diatoms (class Bacillariophyceae, Phylum Chrysophyta) are amongst the most important and most numerous eukaryotic aquatic microorganisms alive. They are extremely abundant both in plankton and sediments in marine and freshwater ecosystems and because they are photosynthetic are very important sources of food for marine organisms. They are often referred to as microalgae and have an extensive fossil record going back to the Cretaceous. Most are less than half a millimetre in size but their oil rich silica shelled bodies sinking to the ocean floor have over long periods of time been transformed into valuable oil and petroleum deposits. The siliceous skeleton is often described as structured like a pillbox or Petri dish with a valve view (from the top) and side or girdle view. Most are symmetrical in shape and often rectangular when viewed from side view. The photosynthetic pigment of diatoms is brown and most diatoms have no visible means of locomotion. In order for diatoms to grow they need to attach to substrate or other algae. Diatoms secrete a form of mucus for locomotion or attachment. Known types of mucilage that diatoms secrete include polysaccharide mucilage, mucilage used in attachment and secreted by

ocellus or mucilage secreted by chitin fibrils thought to be used for deterrence, colony formation or buoyancy (Graham and Wilcox, 2000). The diatom mucilage substances in some cases are thought to help with macroalgae sporling settlement. “The attachment and germination of algal spores are greatly enhanced by excreting mucilaginous substances in intimate contact with the substratum” (Huang & Boney, 1983). Some other toxins are produced but very little is actually known about the chemical nature of all of these or of the diatom mucilage substance (Huang & Boney, 1983)

The relationship between Diatoms and Seaweeds is clearly important and even in the short article by Edsbugge (1966) the relationship between epiphytic diatoms and certain macroalgae was discussed and understood however very little is known about the exact nature of this relationship. In contradiction it has been shown by Huang & Boney that as well as important for settling in some cases certain diatoms can have an inhibiting effect on growth of sporlings. Huang & Boney researched into whether the presence of particular diatoms was an inhibiting factor in the growth of algae sporlings in particular *G.stellata* and *C. crispus*. Their results showed that *G.stellata* has a reduced survival and growth rate in the presence of diatoms *Stauroneis constricta* & *Nitzschia closterium*. *S.constricta* can cause actual physical damage to sporlings. Growth inhibition in *G.stellata* sporlings however was shown only to incur when settling and growing in medium filtrated from stationary and declining phase of SC rather than from exponential phase.

This investigation will test the inhibitory effects of the diatoms *Cocconeis stauriformis*, *Stauroneis constricta*, *Nitzschia closterium* and no diatoms on GS sporlings. The investigation will also test which exact stage of *S.constricta* (i.e. exponential, declining, or stationary) has a significant inhibitory effect on the *G.stellata* sporlings.

S.constricta is the largest of our diatoms and is a freshwater diatom in the Biraphidineae group of which *N.closterium* is also a member although it is a freshwater/marine diatom. *C.stuariformis* is a member of the Monoraphidineae group and is a freshwater/marine diatom. All are periphytic diatoms.

Results

G.stellata was first grown in a control treatment with no diatoms present and then in the presence of the three specific diatoms (*Cocconeis stauriformis*, *Stauroneis constricta*, *Nitzschia closterium*) for 14 days. All sporlings were approximately the same diameter at the start of the experiment and were 40 days old. Table 1 shows the diameter size (um) of *G.stellata* sporling at the end of the 14 days. Net increase in sporlings diameter (um) was measured.

The mean diameter ranged from 211 um where *G.stellata* sporlings were grown with no diatoms present to 175 um where the *G.stellata* sporlings were grown in the presence of *S.constricta*. Testing the data for normality (after standardising the data to residuals from their respective means) showed that the data was not normally distributed (Anderson-Darling $A^2 = 8.834$, p-value = <0.001). Test for heterogeneity of variance on data (performed on residuals) however showed data to be of equal variance. (Levene's test statistic = 0.03, p-value = 0.992. Levene's statistic is used because the data is not normal.) The standard deviations for the sporling diameters grown in the four different treatments are similar (coefficient of variation ranging from 9.7% in treatment with no diatoms to 12.3% in treatment with diatom *S.constricta*).

An analysis of the significance of the data was done using the Kruskal Wallis test, which assumes equal variance but does not assume normal distribution. The Kruskal Wallis test gave an H-statistic of 22.15 and an associated p-value of <0.001 . As this p-value is less than 0.05 we can say that at least one of our sample medians differs from at least one other but we cannot tell which these are. To test for which treatments result in this significant difference the medians were tested using Dunn's procedure.

Table 1: The results and analysis of 40 day old *G.stellata* sporling diameter size (um) for sporlings grown in four different treatments for 14 days.

a) Medians, Standard Deviations (SD) and 95% confidence interval. Where CS = *Cocconeis stauriformis*, SC = *Stauroneis constricta*, NC = *Nitzschia closterium*.

Treatment	Median Diameter	SD	95% CI on Median
No Diatoms	205.00	20.55	201.75 – 208.88
CS	175.00	21.67	171.75 – 178.88
SC	167.00	21.47	163.75 – 172.00
NC	173.00	21.30	169.75 – 176.88

b) Multiple comparisons of average ranks using Dunn’s procedure. Values given by dividing difference of standard deviation. Minimum difference required for significant difference at 5% level = 2.64 um * = significant.

Treatment	No diatoms	<i>Cocconeis stauriformis</i>	<i>Stauroneis constricta</i>
<i>Cocconeis stauriformis</i>	2.46196		
<i>Stauroneis constricta</i>	4.59461 *	2.13265	
<i>Nitzschia closterium</i>	3.15193 *	0.68998	1.44268

The multiple comparisons using Dunn’s procedure shows that there is a significant difference between the no diatom treatment and the *Nitzschia* treatment also a significant difference between the no diatom treatment and the *Stauroneis* treatment. The diameter of sporlings significantly declined in size over the 14 day period when grown in the presence of either *S.constricta* or *N.closterium*.

In the second stage of this experiment 10 day old *G.stellata* sporlings were grown in four separate treatments for a period of 14 days. The treatments were artificial seawater for medium growth, filtrate from *S.constricta* at exponential phase, stationary phase and declining phase. All *G.stellata* sporlings were approximately the same diameter at the start of the experiment. The mean diameter ranged from 108.4 for the sporlings grown in artificial sweater to 90.1 for the sporlings grown in *S.constricta* filtrate at stationary phase. All means were similar to the estimated medians (see Table 2 a). Testing the data for normality (after standardising the data to residuals from their respective means) showed that the data was normally distributed

(Anderson-Darling $A_2 = 0.41$, p-value = 0.329). Standard deviations were similar with coefficient of variation ranging from 5.3% in stationary phase to 6.3% in artificial seawater.

Test for heterogeneity of variance on data (performed on residuals) showed data to be of equal variance. (Bartlett's test statistic = 2.27, p-value = 0.519. Bartlett's statistic is used because the data is of normal distribution).

Table 2: The results and analysis of 10 day old *G.stellata* sporlings diameter size (um) for sporlings grown in four different treatments.

a) Means and standard deviations of *G.stellata* sporling diameter size (um)

Treatment	Mean Diameter	SD
Artificial Seawater	108.4	6.79
Filtrate Exponential Phase	99.5	4.79
Filtrate Stationary Phase	90.1	4.28
Filtrate Declining Phase	94.1	4.77

b) Analysis of Variance Table showing F statistic with associated P-value.

Source	No of treatments	Sum of Squares	Mean Square	F statistic	P-value
Treatment	3	1880.28	626.76	22.78	<0.001
Error	36	990.7	27.52		
Total	39	2870.98			

c) Multiple comparisons between mean diameter size using Tukeys method. All differences in um. Minimum difference required for significant difference at 5% level = 6.32 um (95% CI of the differences). * = significant. Column means are subtracted from row means.

Treatment	Artificial Seawater	Declining Phase	Exponential Phase
Exponential Phase	-8.9 *	5.4	-9.4 *
Stationary Phase	-18.3 *	-4	
Declining Phase	-14.3 *		

Results were analysed using a single classification analyses of variance with results shown in Table 2 b. The p-value from the analysis of variance table was less than 0.05 therefore further analyses of mean differences were carried out using Tukey's method (see Table 2 c). The results of our Analysis of Variance table tell us that at least one of our means differs significantly from at least one other although the standard deviations are not significantly different. A pairwise comparison using Tukey's will help to determine which means differ significantly from which others.

The *G.stellata* sporlings grew significantly more slowly with filtrate taken from all the phases of *S.constricta* in relation to the *G.stellata* sporlings grown in the treatment containing artificial seawater over the 14-day period. *S.constricta* does therefore appear to have an inhibitory effect on the *G.stellata* sporlings and they are not affected by artificial seawater as a growth medium. Further analysis on the mean differences showed that the greatest significant inhibitory effect on the *G.stellata* sporlings was those grown in the filtrate from the stationary phase of *S.constricta* rather than the exponential or declining phases although all three phases showed a significant effect compared with artificial seawater.

Discussion

Laboratory experiments such as these conducted on the growth of *G.stellata* sporlings cannot exactly replicate conditions in the real world (simulate nature) however some interesting findings have been observed from our results.

When *G.stellata* sporlings were grown in the presence of no diatoms and the diatoms *C.stauriformis*, *S.constricta* and *N.closterium* it is clear to see that the presence of the diatom *C.stauriformis* had no significant effect on growth of the sporlings. The diatom *S.constricta* however caused a significant inhibition in the growth of *G.stellata* sporlings. All phases appeared to inhibit growth although growth was most affected during the stationary phase. Growth of *G.stellata* sporlings was more affected by the declining phase filtrate than the exponential phase filtrate indicating that whatever it is that the diatoms do to inhibit growth gets steadily worse through the exponential phase is worse during the stationary phase and although growth rate is not as bad during the declining phase as during stationary it is still a significant effect and much worse than during the exponential phase.

Investigations undertaken by Huang & Boney, (1983, 1984 and 1985) show that the diatom *S.constricta* in stationary phase does have an effect on the growth of *G.stellata* sporlings.

In a study on the effects of diatoms on growth of *Fucus spiralis* (Schonbeck and Norton, 1978) it was concluded that diatoms 'adversely affect the growth of fucoid embryos in culture, clearly *G.stellata* is not alone in being affected by diatoms. The reason for this may be the result of competition for nutrients, light or possibly the excretion by the diatoms of biologically active substances which are either directly antagonistic or operate indirectly by affecting nutrient availability (Schonbeck and Norton, 1978). Organisms compete for attachment and living space and factors that give a competitive edge are an advantage. Sporlings of algae whose growth ensures that they succeed better in this competition clearly have an advantage and since diatoms have rapid growth rates they can quickly out distance competitors in this fight for limited space, light and nutrients. *G.stellata* in particular because of its discoid encrusting sporling phase remains close to the substratum and are likely to be prone to overgrowth by diatoms. Slowing of the centrifugal growth of the discoid sporlings

delays the production of the erect branching system which is also the site of reproduction as well as growth (Huang & Boney, 1984)

When *G.stellata* sporlings are grown in the presence of diatoms sporlings may become abnormal showing a spherical mass of overlapping cells and no sign of branch formation (Huang & Boney, 1983). Further investigation would be needed to investigate what the reasons are for inhibition of growth and fitness and if it is an excretion of some sort possibly connected to the mucilage excretions of diatoms exactly what chemical it is that affects growth as little is known about the chemical nature of diatom mucilage at present (Huang & Boney, 1983). *S.constricta* was observed by Huang & Boney (1984) to inflict actual physical damage on *G.stellata* with older sporlings with dense growths of diatoms resulting in loss of uppermost cell walls and exposure of cell contents.

Diatom epiphytes on other algae also show contradictions in results with some algae being affected others not and some algae and diatom species benefiting from interaction. In a study of diatom settling on *Odonthalia floccose* (a red algae) increasing levels of epiphytes were correlated with declines in host photosynthesis, growth and reproduction. Experiments confirmed that reduced growth was due to diatoms. This research clearly showed that for some algae especially those that obtain light and nutrients through the thallus epiphytes created a competition for resources, which resulted in inhibiting effects of the diatoms on the algae (Ruesink, 1998)

Huang and Boney's research also included examining residual diatom mucilage and its growth inhibiting factors. It was shown that the residual mucilage could have a marked effect on morphogenesis of certain sporlings. All three of the diatoms in our experiment produce a slime film although in the case of the larger *C.stauriformis* the mucilage is only produced through the raphe on one valve rather than on both valves, as is the case with *S.constricta* and *N.closterium*. The growth substances that were contained in the residual mucilage controlled growth development including cell division, morphogenesis and oranogenesis (Huang and Boney, 1983).

The toxin produced by *Pseudo-nitzschia* could be a possible factor. This toxin has been proven to effect shellfish causing amnesiac shellfish poisoning in humans but no

link has been made with this toxin and growth of algae. The toxin 2-trans, 4-trans decadienal (also known as DDE) produced by *N.closterium* could also be a factor although there is no evidence to link this with algae and this does not explain the effect of *S.constricta* on *G.stellata*.

When considering the effects of diatoms on algae it is worth considering the class and phylum of the individual diatom, its biology (size etc) and its natural habitat as factors. The diatoms requirement for nutrients like silicate, carbonate, Zinc, Iron or nitrate are also factors. Competition for the same nutrients could arise. Alternatively a state of eutrophication could occur which is known in some cases to block out sunlight to plant life, which would be unable to photosynthesis properly.

It is certain given our results that *S.constricta* does something that inhibits *G.stellata* growth increasingly during the exponential phase. When in stationary phase (bloom) growth is severely inhibited and growth continues to be inhibited in the declining phase although slightly less so than during the preceding stationary phase. The damage has already been done. It is however difficult without further research to ascertain exactly why this occurs.

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