The Inhibition of *Sargassum muticum* by Different Macroalgae

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Marine algae course

Summer 2012

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Abstract

Allelopathy in plants is a known phenomenon that describe chemical defense against other plants. Lately there are studies that describe this phenomenon in the marine environment but most of the study don't concentrate on the macroalgae-macroalgae relationship. In this study I tried to learn how different native algae influence on the *Sargassum muticum*'s growth. I found out that under stress the *Sargassum*'s growth can be inhibited by the *Ulva californica* and *Fucus distichus ,* but not by *Pyropia kanakaensis.* I also tried to determine whether the snail *Lacuna vincta* eats the *Sargassum* itself or just the diatoms on the surface. My very preliminary results show that the *Lacuna* prefer to eat the diatoms.

Introduction

Sargassum muticum is native to the north-western Pacific and is found in the coastal waters of Japan, China, Russia, and Korea. It was first observed outside of its native range in British Columbia, Canada in 1944. At present, *Sargassum muticum is* established in North America on the eastern Pacific coast from south-eastern Alaska in the north to Baja California, Mexico in the south (Wallentinus 1999). *S. muticum* occupies lower intertidal and subtidal habitats, and forms a conspicuous component of the intertidal macroalgae owing to its large size and radial morphology.
The macroalga *Sargassum muticum* (Yendo) is one of the most aggressive marine invaders (Boudouresque and Verlaque, 2002; Norton, 1976). *Sargassum muticum is* considered a pest and fouling species, which interferes with recreational and commercial use of waterways (Critchley et al.1986) particularly when it becomes detached from holdfasts and forms large floating masses (Farnham et al.1981). On the other hand, other studies show that sometimes if there are strong native algae they inhibit the invasive species growth, for example Dong et al. (2012) show in their study that although *U. prolifera* is the causative species of the world’s largest green tide and its blooms have major ecological and economic impacts, the presence of a stable native algal canopy of *G. lichvoides* may inhibit its expansion.

A handful of studies have shown or suggested that extracts from *Ulva* spp. inhibit larval development in barnacles, crab, and flounder (Magre 1974, Johnson 1980, Johnson and Welsh 1985). Further, extracts from *Ulva pertusa* Kjellman and other macroalgae have been shown to have inhibitory effects on dinoflagellates (Jeong et al. 2000).

Allelopathy, which is one type of direct plant competition, can play an important role in ecosystem structure and plant diversity (Blunt 2010). Although the importance of allelopathy as a mechanism of competition is gaining prominence in terrestrial ecological research, the importance of allelopathy in aquatic ecosystems has received less attention, especially among macroalgae (Macı´as 2008). Recent study illustrate that the native macroalgae *G. lichvoides had* strong allelopathic effects on the opportunistic species *U. prolifera* (Dong et al 2012).

In this study I wanted to see if different genera of algae will affect on the growth and spread of the invasive species algae, *Sargassum muticum*.

The release of toxic compounds from *Ulvaria* was tied to desiccation for periods of time that ordinarily cause death in this species (Nelson 2000) and for that reason I dried the four algae before I added them to the *Sargassum muticum* algae to see if their chemical defense will inhibit *Sargassum*'s growth. Nelson (2003) found in his study that *Ulva* and *Ulvaria* extract treatments had a significant impact on germination frequency and germ tube length of *Fucus* zygotes. Therefore, I checked the influence of the extracts of four the different algae on the zygotes of the *Sargassum muticum*.

I also tried to reject the claim that *Sargassum muticum* is the preferably food of *Lacuna vincta* (Britton-Simmons et al, 2010) and that it isn't the factor that mediate it's growth. I show that *Lacuna vincta* probably eat the diatoms from the surface of *Sargassum muticum*. Padilla (1998) determined in her paper that the genus *Lacuna* has plastic tooth morphology meaning they can change the morphology of their teeth depend on the food they have.

Methods

Can *Sargassum muticum* be inhibited by different macroalgae?

In order to answer this question I dried out a *Sargassum muticum* plant for 24 hours in the sun until it was totally dry and put it in 5 different flasks (100 ml) each one with one of the three different algae and another one is the control. The algae I chose to work with were *Ulva californica, Pyropia kanakaensis* and *Fucus distichus.* I chose these algae because they are very common in this area and they represent the 3 different phyla: green, brown and red. The green algae are represented here by *Ulva californica* which release toxins under stress. I measured mass and volume of each alga.

The dead *Sargassum* stayed in the flasks with the different algae in order to cause stress so they will activate their defense and release chemical into the water (allelopathic reaction).

After 24 hours I took all the algae out, I dried the four algae causing them an extra stress. When the algae were dry I chopped them and added them to their older flask (each alga to the flask it was in first time because it might release some chemical as a reaction to the exposure to the *Sargassum*). To each flask I added a piece of stipe with one branch from the *Sargassum* (the stipe is important for the length measurements and the branch to an effective photosynthesis). I measured wet mass and length with digital caliber. I repeated those measurements on day 3 and day 6 of the experiment (I also took measurements on day 11 but most of the algae were dead so the results are not reliable). The flasks were aerated for the first three days and when I saw the algae were dying I stopped the ventilation. The flasks were kept outside (for the sunlight) and in tank that covers half of their height so it keeps the temperature the same as at the ocean.

Can *Sargassum muticum* zygote germination be inhibited by different macroalgae?

I did the same procedure as at part 1 but now instead of adding pieces of *Sargassum* I added its gametes. To get it's gametes I dried it for 24 hours and then took the reproductive parts, wet them and the gametes released.

After 10 days checked if the gametes reproduced, made zygotes and the zygotes germinated and if they did how long is their germ tube compared to the control where there were just gametes.

Does *Lacuna vincta* mediate *Sargassum muticum* growth

I collected 15 individuals of the snail *Lacuna vincta* and locked them in a cage cover with thick net, so they can't go out, inside the tank to keep them wet and in the right temperature. I starved them for 16 hours and then I added them stipe with branches from the algae *Sargassum muticum*. As a control I used a *Sargassum muticum* with no snails on it at locked it in a similar cage. After 16 hours I looked at the *Sargassum muticum* using SEM and pictured the two pieces of the *Sargassum.*

Results:

The growth of *Sargassum muticum* inhibited by others macroalgae.

At the first three days the flasks were aerated and the rate growth of the treatments and the control was negative (Fig 1a). At day 6, after I stopped the aerating and changed the water in day 3, the *Sargassum*'s growth in the flasks with *Ulva californica* (it is not significant $p<0.08$, $N=6$) and *Fucus distichus* (it is not significant $p<0.09$, $N=3$) was inhibited (Fig 2a,b). The *Sargassum*'s growth in the flasks with the *Pyropia kanakaensis* wasn't inhibited (Fig 2a,b). Additionally visually the *Sargassum* were in the flask with the *Ulva* and the *Fucus* looked very bad compared to the control (Fig 1 in appendix).

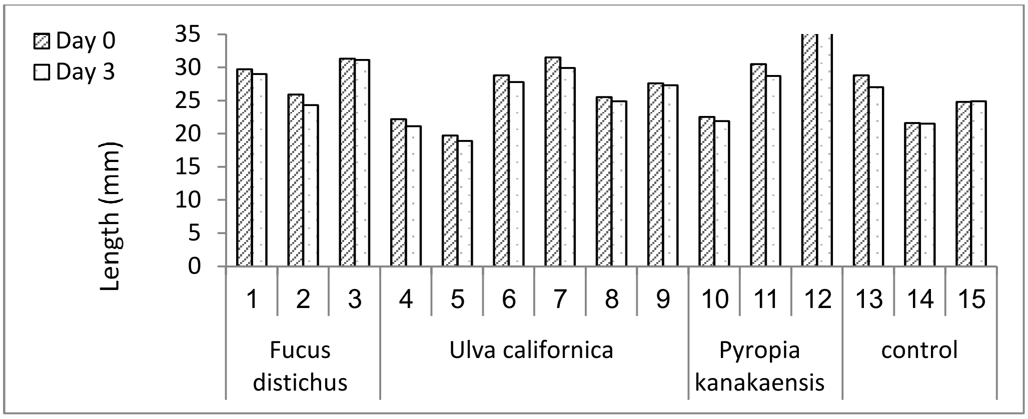


Fig 1a The length (mm) of *Sargassum muticum* growing with *Fucus distichus, Ulva californica* and *Pyropia kanakaensis* on day 0 and day 3.

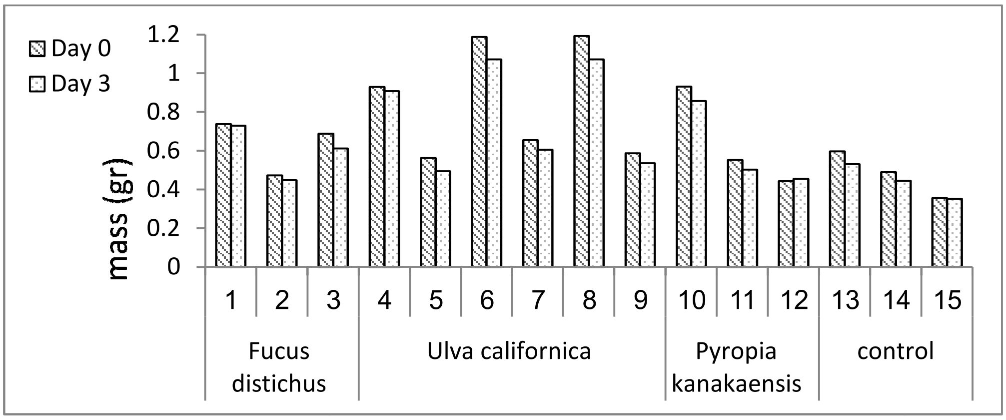
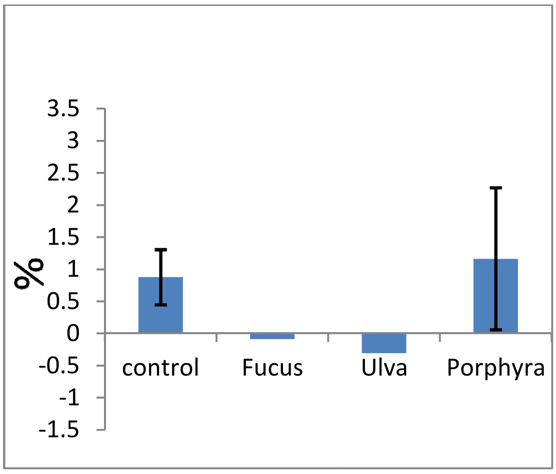


Fig 1b The mass (mm) of *Sargassum muticum* growing with *Fucus distichus, Ulva californica* and *Pyropia kanakaensis* on day 0 and day 3.



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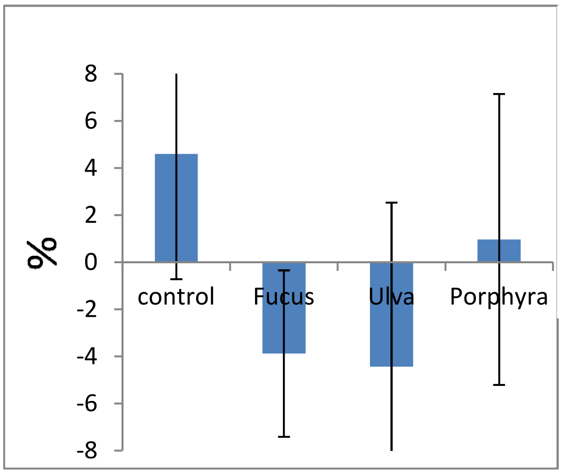

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us (N=3
N=6 p<0.08)
=3) from day

In the pictures taken with SEM when the *Sargassum* was without *Lacuna vincta* in the cage there are groups of diatoms along the stip and the branched of the *Sargassum* (Fig 3a,b, appendix Fig 2), while at the *Sargassum* that was in the cage with the *Lacuna vincta* you can see mostly isolated diatoms and the stipe and branches look whole (Fig 4a, b).

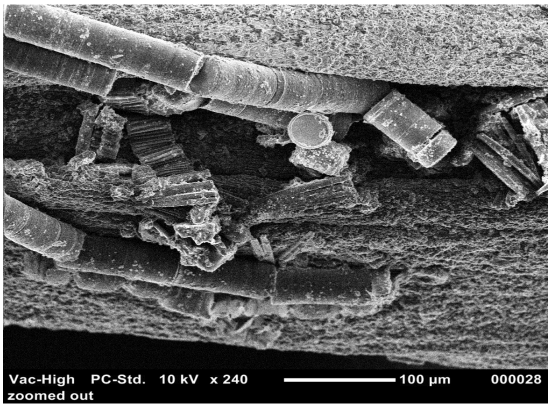

Fig 3a SEM picture of *Sargassum* without *Lacuna vincta*

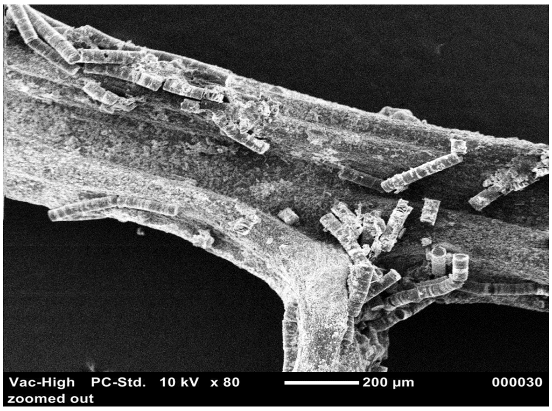

Fig 3b SEM picture of *Sargassum* without *Lacuna vincta*

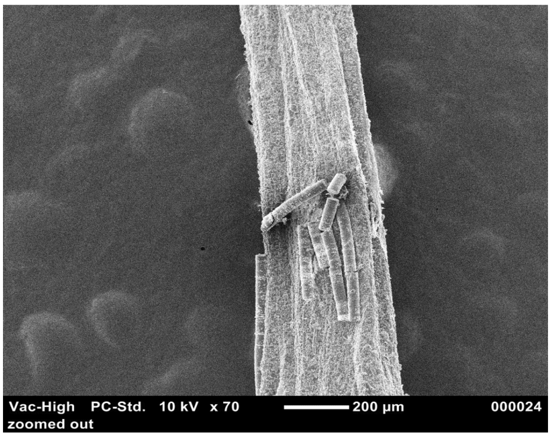

Fig 4a SEM picture of *Sargassum* with *Lacuna vincta*

Fig 4b SEM picture of *Sargassum* with *Lacuna vincta*

Discussion:

My results suggest that *Sargassum muticum* growth can be inhibited by *Ulva californica* and *Fucus distichus* but not by *Pyropia kanakaensis*. I showed that while the *Sargassum* in the control and with the *Pyropia* continued growing the *Sargassum* with the *Ulva* and *Fucus* were dying (Fig 2a,b). The results not significant probably because the sample was small (N=3). In addition measurements of mass and length don't express very well the differences between the algae, because the dead algae don’t gain mass and length but their lost of mass and length limited. Another problem was that at the first three days the flasks were aerated and it was probably too strong to those small flasks and the whirling was also too strong and it damaged all the algae (Fig 1a,b). That is the reason my results are limited to stressed *Sargassum,* I can't tell what would be the results if the only stress to the *Sargassum* was the native algae that were in the flasks with it.

Comparing the pictures of the *Sargassum* with and without the *Lacuna* I can suspect that the *Lacuna* is eating the diatoms from the surface of the *Sargassum* and not the alga itself because I didn't find any wounds on the *Sargassum* surface (Fig 4a) furthermore, on the surface of the *Sargassum* without the *Lacuna* I could see big groups of diatoms (Fig 3a,b) while on the surface of the *Sargassum* without the *Lacuna* I couldn't find one.

- The gametes didn't create zygotes so I don’t have results to the second part of my study.
- The SEM broke dawn so I couldn't repeat the experiment of the *Lacuna* food preference so I don’t have any statistics.

Conclusions:

The area of allelopathy at marine environments and in macroalgae specially is unknown, just in the last years people start to investigate it. My study just came to show an idea that should be study in the future. For future studies at this area it is important to separate the different stress factor. In my study I caused the three native algae three different stresses: first, when I collected them straight to the flasks, second when I put them with the dead *Sargassum* in the same flask, and third, when I dried them in the sun. In order to determine if there is allelophtic reaction I need to neutralize all the other effects but the *Sargassum* effect. Another thing to think about is that bacteria that live on the algae that can cause the death of the *Sargassum*.

The results of the *Lacuna* food preference are very preliminary and I need not just to repeat them and do a statistic but also to try to starve them for longer time and to give them more time to eat the same stipe of *Sargassum* so I can really say that this is what they eat because as I mentioned before *Lacuna* have plastic teeth morphology and the new grow respectively to the food it has.

Acknowledgments For helpful discussions about this work and a lot of patience I thank Tom Mumford and Bob Waaland my professors and Katie Dobkowski the T.A. For the scholarship that helped me to get here I thank all the generous donors and FHL contact people especially Stacy Markman. I also want to thank all my friends from the course, you are really amazing group!! To all my lab members from Israel and of course to my mother and sister that supported me all the way.

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Appendix

Fig 1

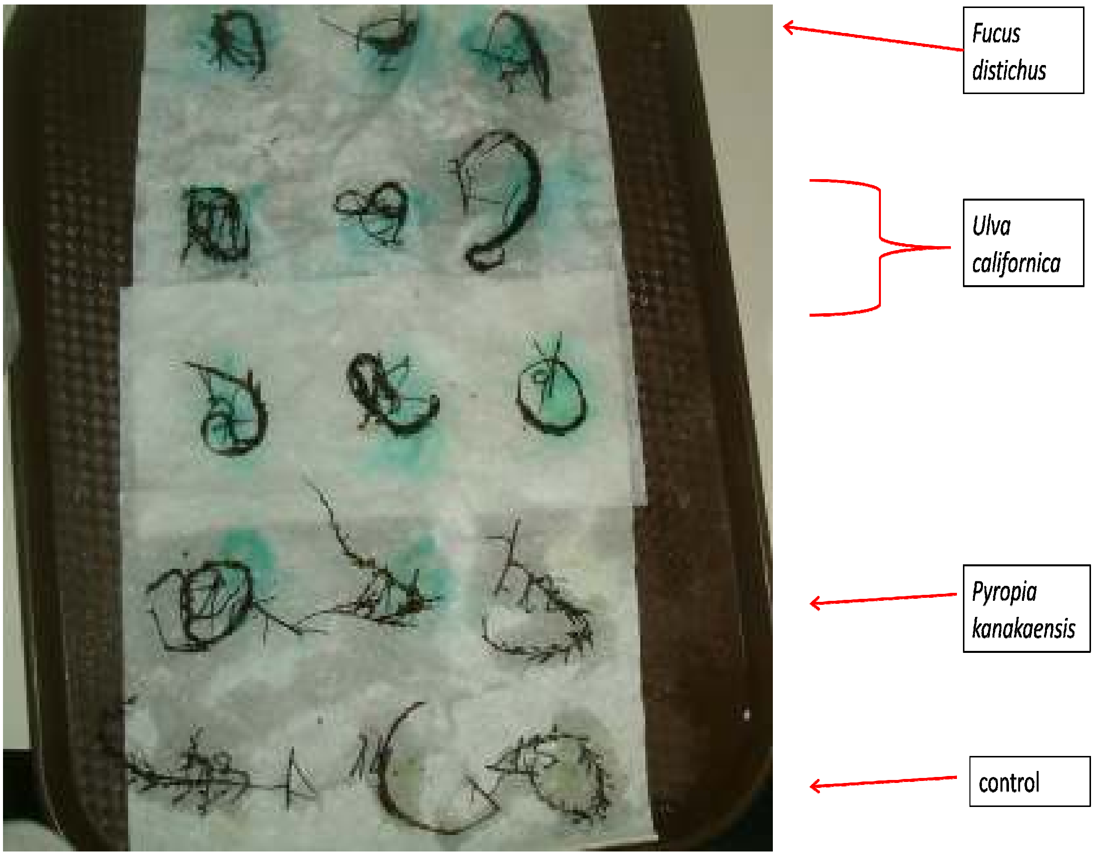
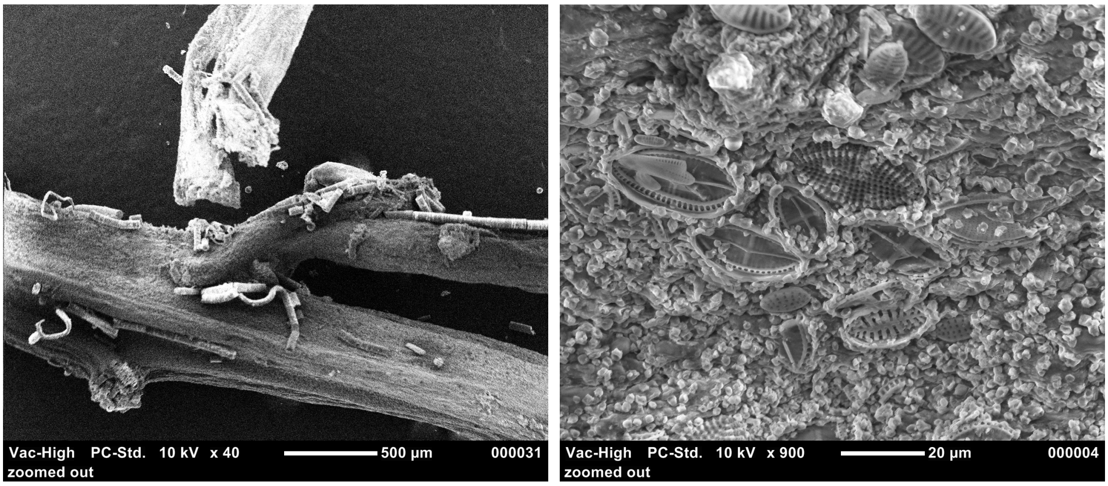


Fig 2a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