Kelp gametophyte recruitment in articulated coralline algae of the San Juan Islands

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Abstract

Kelp species dominate rocky intertidal communities in the Salish Sea and overlap in distribution with a diverse array of articulated coralline algal species. Little is known about the microscopic gametophyte phase of kelp's biphasic life cycle. Reproductive success and maturity of both life phases is crucial to kelp persistence, and it has been suggested that coralline algae may play a role in kelp gametophyte settlement and recruitment. To test whether *Hedophyllum sessile* gametophytes settle in the genicula of coralline algae species (*Bossiella plumosa*, *Corallina vancouveriensis*, *Calliarthron tuberculosum*), we collected coralline samples near *H. sessile* populations and examined their genicula under a microscope. Those assumed to have brown algal gametophytes underwent DNA extraction, amplification, and PCR using the universal plastid amplicon (UPA) and internal transcribed spacer (ITS) regions. Samples that amplified with ITS primers were sequenced but results were inconclusive. To complement the field study, a tank experiment was used to investigate *Alaria marginata* spore settlement. One week after spores were released into treatment jars (control, algae+spores, algae, autoclaved algae+spores), thalli and settlement plates were examined for gametophyte recruitment. We extracted and amplified DNA from those with growth, performed PCR using the UPA and ITS regions, and sequenced those that amplified. The control jar with spores and no algae contained the most actively-growing and developed gametophytes, while the jars with coralline algae had underdeveloped or dead spores. Future studies should investigate whether coralline toxins may inhibit settlement and recruitment of algae in coralline genicula.

Introduction

About twenty-three kelp (Phaeophyta, Laminariales) species dominate rocky intertidal and subtidal algal communities in the Salish Sea, where they overlap in their vertical distribution with a diverse array of articulated coralline algal species (Parada et al. 2017). Coralline algae (Corallinaceae) are a calcifying family of red algae whose morphological diversity includes encrusting, articulated, and epiphytic forms that contribute to calcium carbonate sediment accretion and act as a significant nearshore carbon store, with production rates similar to salt marsh, sea grass, and mangrove ecosystems (van der Heijden & Kamenos 2015). It has been suggested that coralline algae may play a role in kelp gametophyte settlement and recruitment, but the ecological and chemical nature of that relationship is still unknown (Parada et al. 2017).

Kelp species have a life cycle that alternates between a diploid sporophyte phase and a haploid gametophyte phase (Liu et al. 2017). While kelp sporophytes are visible to the human eye, making them accessible to study, little is known about the haploid gametophyte phase of kelp's biphasic life cycle (Fox & Swanson 2007, Bartsch et al. 2008). Kelp gametophytes are microscopic and filamentous, and often resemble other small filamentous brown algal species, making them difficult to identify and observe in situ on intertidal substrata (Bartsch et al. 2008). Laboratory experiments have often been used in an attempt to observe and identify gametophytes, but can fall short of predicting the settling and recruiting habits of kelp gametophytes within a natural ecosystem setting (Bartsch et al. 2008).

Recent studies dispute whether and how coralline algae regulate the settlement and recruitment of other species in their genicula. A 2017 study by Parada et al. found that *Lessonia spicata* (Order Laminariales) spores preferentially settled in the genicula of articulated coralline

algae, while crustose coralline algae inhibited spore settlement. While some studies have found that coralline algae emit chemical cues that chemotactically attract the larvae of select herbivorous invertebrates (Roberts et al. 2004), it has also been suggested that corallines use strategies to inhibit the recruitment of macroalgal gametophytes in their genicula, either by sloughing their epithallial tissues or by releasing allelochemicals (Kim et al. 2004). Most studies agree, however, that the relationships maintained by coralline algae with organisms settling in their genicula are largely species-dependent. Therefore, it is likely that the abundance of kelp gametophytes in intertidal ecosystems may be influenced by the presence of coralline algae.

Kelp and coralline algae may be threatened by rising ocean temperatures, which are expected to increase 1.5 ºC by 2095 in the Salish Sea, due to anthropogenic forcings (Bartsch et al. 2008, Khangaonkar 2019, Berry et al. 2021). Warming ocean temperatures above 16 ºC can inhibit photosynthesis in the macroscopic, diploid sporophytic phase of the kelp biphasic life cycle, whose optimal temperature for growth and development is approximately 15 ºC (Paine et al. 2021). Ocean acidification and thermal stress can increase the susceptibility of coralline algae to bleaching (McCoy & Kamenos 2015, Guy-Haim et al. 2020). In order to ensure long term viability of kelp stands, it is critical that reproduction and recruitment are successful in both phases of its biphasic life cycle (Bartsch et al. 2008). Reproduction in kelp gametophytes gives rise to mature kelp sporophytes, which, in turn, reproduce to bear kelp gametophytes. Because of the alternate, biphasic nature of the kelp life cycle, kelp populations cannot persist without the reproductive success and maturity of both its sporophyte and gametophyte life phases. Complications in settlement and recruitment in either of the two phases can lead to the collapse of entire kelp populations (Liu et al. 2017).

Kelp play an ecologically important role in primary productivity, food webs, and carbon sequestration in the Salish Sea (Berry et al. 2021), provide critical ecosystem services in rocky intertidal and subtidal communities, and have promising marine agronomic value (Goecke et al. 2020). For these reasons, we are continuing the study of kelp gametophytes to better understand why kelp populations are currently struggling and to determine what factors may limit future kelp recruitment and recolonization.

Our study focuses on kelp communities of twenty-three species of kelp in the San Juan Islands, a cluster of glacially carved islands in Washington's northern Salish Sea (Fig. 1). The Salish Sea is protected from exposure to the oceanic swell and wind-driven waves of the outer Olympic coastline by the Strait of Juan de Fuca (Bell 1994, Gaylord 1999). Influxes of oceanic tidal currents combine with freshwater inputs to give the Salish Sea and Strait of Juan de Fuca a brackish, estuarine quality (Cannon 1978). Salinity gradients, nutrient fluxes, current exchanges, and low wave action all interact to shape the species composition of Salish Sea intertidal and subtidal algal communities (Jensen & Denny 2016). This large number of kelp species found in Washington is one of the most diverse in the world (Calloway et al. 2020).

Our study builds upon the work of Poulin and Tonra (2018) who investigated *Hedophyllum sessile* (Order Laminariales) gametophyte settlement and recruitment on the genicula and intergenicula of three species of articulated coralline algae, *Bossiella plumosa, Corallina vancouveriensis,* and *Calliarthron tuberculosum*, at Cattle Point and Eagle Cove on San Juan Island. Poulin and Tonra (2018) chose to study *H. sessile* because little is known about its gametophytic phase and it has been found on coralline algae previously (Parada et al. 2017). Poulin and Tonra (2018) extracted DNA from coralline genicula assumed to have brown algal gametophytes, which then underwent PCR of multiple loci specific to *Alaria marginata,*

Costaria costata, and *Nereocystis luetkeana* to determine whether the gametophytes observed were *H. sessile*. The DNA in the corallines did not amplify for any of the primers used, which suggested that the amplified DNA belonged to *H. sessile* (Poulin & Tonra 2018). After sequencing the DNA, Poulin and Tonra (2018) determined that the amplified brown alga DNA belonged to *Scytosiphon sp.*, rather than *H. sessile*. Our study uses modified methods of Poulin and Tonra (2018) to collect samples of coralline algae and extract DNA from gametophytes present in the genicula of coralline algae. We wanted to corroborate the findings of Poulin and Tonra and directed our study towards finding a beneficial relationship between coralline algae and kelp gametophytes rather than investigating potential negative relationships.

To complement our field study that used DNA analysis to identify kelp gametophyte settlement on articulated coralline algae, we set up a controlled laboratory experiment to investigate *Alaria marginata* gametophyte settlement. Our goal was to document the process of gametophyte settlement on coralline algae in a controlled environment in order to better understand how kelp gametophytes settle on coralline algae in the field. While our field study investigated the relationship between *H. sessile* gametophytes and coralline algae, we studied *A. marginata* gametophyte settlement on articulated coralline algae in our laboratory tank experiment. We chose to study *A. marginata* because it becomes fertile in early- to mid-summer, which overlapped with our timing of sample collection (McConnico & Foster 2005) and *H. sessile* was not fertile when we performed these experiments. *A. marginata* is closely related to *H. sessile* as they are both kelp species, have a temperate geographic distribution, and are commonly found in the low intertidal zone near substrata occupied by articulated coralline algae (Druehl & Clarkston 2016). By studying interactions between coralline algae and kelp gametophytes in both field and laboratory settings, we hope to gain a more comprehensive

understanding of the gametophytic phase of kelp and whether coralline algae play a role in their recruitment.

For our field study, we hypothesize that in natural ecosystem settings, the gametophytic phase of *H. sessile* will settle and recruit on all three species of coralline algae collected: *B. plumosa, C. vancouveriensis,* and *C. tuberculosum*. We hypothesize that coralline algae may emit chemical and/or physiological cues that chemotactically attract kelp gametophytes to settle and recruit on the genicula of articulated coralline algae. We hypothesize that dead coralline algae do not emit these cues, and therefore the autoclaved treatment will have the least spore recruitment.

Materials & Methods

Sample collection

Samples of articulated coralline were collected from Cattle Point, San Juan Island (Fig. 1), on June 26th, 2021 during low tide (-2.96 ft at 12:32 pm). For the tank experiment, we chiseled samples off the rock to retain the holdfast and some substrate. The algae collected was within 30 cm of thalli of *H. sessile*. The algae were transported to the lab in seawater half an hour after collection and kept in flow-through sea tables. Samples were identified using Gabrielson & Lindstrom (2018) and sorted into bins based on their species identification, either *Bossiella plumosa, Corallina vancouveriensis,* or *Calliarthron tuberculosum*.

Molecular analysis

The genicula of the three species of coralline algae were examined under a Nikon Eclipse 50i compound microscope (Minato City, Tokyo, Japan). Samples containing brown spheres or

tubes within or on the edge of genicula (Fig. 2) were assumed to be brown algal gametophytes and were separated for further analysis using molecular methods. A control without any visible brown filaments was also sampled.

DNA was extracted and amplified from the samples using the MyTaq Extract-PCR kit (Meridian Bioscience, Cincinnati, OH, USA). Enzymatic solution from the MyTaq kit was added to the coralline samples and incubated at 75°C. *B. plumosa* and *C. vancouveriensis* samples were incubated for 1 hour, and *C. tuberculosum* samples were incubated for 1.5 hours because they were harder to break down. Samples were additionally broken down using tube pestles, and incubated at 95°C for 10 minutes. The OneStep PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA) was used to further clean the DNA extractions, and the resulting DNA extraction solution was diluted to 0.1 of its previous concentration.

Two loci were chosen for PCR. The universal plastid amplicon (UPA) region was selected to verify the presence of amplifiable brown and coralline algal DNA. The UPA primers were p23SrV_f1 and p23SrV_r1 (Sherwood & Presting 2007). The nuclear ribosomal internal transcribed spacer (ITS) region was selected to test for the presence of brown algae using the primers ITS-P1F/KG4 (Druehl et al. 2005). The PCR mixture contained 4.25 µL of distilled water, 0.5 µL of 10 µM forward and reverse primer, 6.25 µL MyTaq mix, and 1.0 µL of the template DNA. Temperature cycling was performed in a T3 Thermocycler (Biometra, Göttingen, Germany) using the following protocol: an initial denaturing step of 95°C for 2:45, followed by 35 cycles of 95°C for 0:15, 45°C for 0:15, and 72°C for 1:00, with a final extension step of 72°C for 5:00. The PCR product was run through a 1.5% agarose gel to verify that DNA had been amplified. Samples that contained ITS PCR product were sequenced in both directions, and 6 samples that contained UPA PCR product were sequenced in one direction. PCR products were

cleaned using illustra ExoProStar (GE Healthcare, Chicago, IL, USA) and were prepped for sequencing by combining 2.5 µL of 10 µM primer, 9.5 µL of distilled water, and 3 µL of PCR product. If the band of DNA on the gel was faint for a sample, 4.0 µL of PCR product was added and only 8.5 µL of water. The premixed sequence reaction preparations were sent to Genewiz (Seattle, WA, USA) for sequencing. Individual reaction results were edited and combined to generate sequences using the computer software Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were compared to NCBI database sequences using a BLAST (Basic Local Alignment Search Tool) nucleotide search.

Tank experiment

We conducted a tank experiment to determine whether the coralline alga *Bossiella plumosa* may emit cues that cause spores to settle on them. Four glass jars containing seawater and four microscope slides were autoclaved at 80℃ for 2 hours using an autoclave SX-500 (Tomy, Katsushika, Tokyo, Japan). The jars were then placed in 11.7℃ seawater tanks that had a water level slightly below the rim of the jars so that there was no exchange of sterilized and unsterilized seawater during the experiment. Each jar was a different treatment. One sample of *B. plumosa* was autoclaved to kill and sterilize the algae, while still allowing the structural elements to remain intact. This was done to prevent any chemical or physiological recruitment cues. Samples of live *B. plumosa* on rocks were placed into two jars. Microscope slides were used as small settlement plates and were placed in each of the three jars. The fourth treatment was the control and only contained a settlement plate (Fig. 3).

Reproductive thalli from *Alaria marginata*, a kelp species with spores similar to *H. sessile*, were collected from the Friday Harbor Labs dock on July 1st, 2021. Thalli were wrapped

in a damp paper towel, refrigerated overnight, then placed in a bowl of water to induce spore release. 100 mL of the spore and water mixture was added to 400 mL seawater containing the autoclaved algae treatment, the control jar, and a jar with live algae (Fig. 2). After one week, the settlement plates from each jar were examined under a Nikon Eclipse 50i compound microscope (Minato City, Tokyo, Japan) to observe the growth/death of gametophytes, and photos were taken. 5 coralline thalli measuring 2 cm tall from each treatment were examined under the compound microscope to identify spores and potential gametophytes using the same criteria as the first experiment. We extracted and amplified DNA from genicula with suspected *A. marginata* gametophytes from each of the five thalli. We used the p23SrV_f1/p23SrV_r1 (Sherwood & Presting 2007), ITS-P1F/KG4 (Druehl et al. 2005), and ITS *Alaria marginata* specific forward/KG4 primer pairs (Druehl et al. 2005). PCR products from reactions with *A. marginata* specific primers were sequenced as described above.

Results

Molecular Results

A total of 6 samples of *B. plumosa*, 6 *C. vancouveriensis*, and 14 *C. tuberculosum* were suspected to contain *H. sessile* gametophytes after examining thalli under the microscope. The UPA primer pair successfully amplified DNA in all of the samples (Fig. 4). The ITS primer pair only amplified DNA in 3 *C. tuberculosum* samples, CA6, CA7, and CA11 (Table 1, Fig. 5). Sequence reactions of CA6 and CA7 both contained multiple DNA fragments and those for CA11 were inconclusive. This made the brown algae potentially found in the genicula unidentifiable (Table 1).

Tank Experiment Results

All of the samples besides algae + spores sample 4 (AS4), AS5, and autoclaved algae + spores sample 2 (XS2) amplified well with UPA primers (Fig. 6). The ITS primers amplified multiple products in AS3 through AS5 and XS1 through XS5 (Table 2). The different and/or more weakly amplified products resulted from reactions with the algae + no spores samples (A1-A5) and AS1-AS2. AS2-AS5 and XS1 amplified with the *A. marginata* specific ITS primers well, and AS1 amplified faintly (Fig. 6).

The control jar with no algae and spores contained the most actively-growing gametophytes on the settlement plate (Fig. 7d). Additionally, this settlement plate had the most developed gametophytes. The autoclaved algae with spores had the highest concentration of spores out of the coralline algae treatments, and also contained the most diatoms (Fig. 7c). The spores (3-5 µm in diameter) appeared to have not germinated (no germ tube visible) and to be dead in the coralline algae treatments (Fig. 7b and 7c).

Discussion

Coralline algae toxicity

Our study demonstrates the existence of a relationship between kelp gametophytes and articulated coralline algae. The nature of this relationship is not as we expected; instead of coralline algae chemo-attracting kelp gametophytes to live endophytically in their genicula, we have reason to believe coralline algae may release a toxin that partially inhibits *Alaria* zoospores and gametophyte settlement and recruitment.

Our DNA sequence results from our molecular analysis of our field experiment were inconclusive and we were unable to determine what species of algae may be living in the

genicula of the three species of coralline algae that we collected from the field. We did, however, observe organisms, such as filamentous red algae, living endophytically (Fig. 2) in the genicula of the articulated coralline algae during our microscope surveys. It has been shown that coralline algae chemotactically attract larvae of herbivorous invertebrates, such as *Haliotis iris* (abalone), and quickly induce larval metamorphosis (Roberts et al. 2004), so it is possible that the organisms we observed were invertebrate larvae or other microorganisms and not brown algae. Another possibility is that the coralline algae regulate their chemical cues to both attract beneficial herbivorous invertebrates and attempt to inhibit the growth of macroalgal gametophytes, which may outcompete the coralline algae for light and nutrient resources.

This latter statement is supported by our settlement plate results from our tank experiment. We observed the highest density and most developed gametophytes of *A. marginata* on the settlement plate in the jar without coralline algae. In the jars with coralline algae, the *A. marginata* spores were either dead or underdeveloped (Fig. 7), suggesting that the presence of coralline algae had a negative effect on *A. marginata* spore growth and development.

A Contradicting Result

Our tank experiment PCR results contradict our settlement plate result. The DNA extracted from the genicula of both living and autoclaved coralline algae in the tanks with spores amplified with the *A. marginata* specific ITS primers (Fig. 6), suggesting that *A. marginata* gametophytes had settled in the genicula of those specimens of *B. plumosa*. It is possible, however, that the DNA amplified was extracted from dead *A. marginata* gametophytes/spores, and further studies should consider this possibility when designing a similar assay. While the distinct differences in spore concentration and development between settlement slides suggest

coralline inhibition of kelp gametophytes, our DNA amplification/PCR results from the extracted algal tissue argue against the possibility of such inhibition. Our conflicting results indicate the need for further replications of the tank experiment.

Limitations

Our molecular results may have been affected by the size of the coralline thalli pieces we extracted. They may have been too large, and the DNA from the brown algal gametophytes may have been overwhelmed by coralline DNA. This would explain the multiple DNA sequences found in many of the sequence reaction results. Future experiments should address this by extracting a variety of thalli sizes to amplify. Additionally, amplified DNA with clean sequence results were only sequenced from one direction, making it difficult to identify to a genus or species level.

There were several limitations to the tank experiment. We were unable to chisel enough rock pieces to have more replicates. This also limited how many species we could use and ultimately only *B. plumosa* was used in the tank experiment instead of all three coralline species. Ideally, each treatment would have at least five replicate jars with a few thalli on each rock and each of the three coralline species could be used in the treatment assays. Because we did not want to keep algae out of the water for an extended period of time before the experiment, we were not able to observe spores or gametophytes that may have been already growing in genicula before treatments. At the end of the week, the water in the jars with the algae + spores and the autoclaved algae + spores smelled and could have been a sign that the water had gone bad. This may have led to the spore death we observed. If we repeated the experiment, we would allow the spores to settle, then change the water in the jars periodically.

Future directions

The tank experiment could be repeated in the future with more replicates to expand on our results. We could also add a control with no corallines and no spores, to be sure there is no contamination in the jars. For our experiment, we only used *B. plumosa*, and we could repeat the experiment with *C. vancouveriensis* and *C. tuberculosum*. This could help determine whether *B. plumosa* is unique in potentially repelling kelp gametophytes, or whether it is common in other species as well. A further experiment could measure chemicals produced by corallines, and whether autoclaving prevents these chemicals from being released.

A study by Parada et al. (2017) demonstrated that crustose coralline algae inhibited kelp spore settlement, but kelp spores readily settled in the genicula of articulate coralline algae. Given that our findings do not fully align with the work of Parada et al. (2017), and that we did not study crustose coralline algae, future work on the inhibitive relationship between coralline algae and kelp should include the study of encrusting coralline species.

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Table 1. PCR and sequence results from the first experiment.

ID #	Species	Sample #	UPA?	ITS?	Sequenced?	Result if sequenced?
B1	*B. plumosa*	1	✓			
B2	*B. plumosa*	2	✓		✓	Clean sequence
B3	*B. plumosa*	3	✓			
B4	*B. plumosa*	4	✓			
B5	*B. plumosa*	5	✓		✓	Clean sequence
B6	*B. plumosa*	6	✓		✓	Poor- maybe 2 sequences
C1	*C. vancouveriensis*	7	✓			
C2	*C. vancouveriensis*	8	✓		✓	Poor- maybe 2 sequences
C3	*C. vancouveriensis*	9	✓			
C4	*C. vancouveriensis*	10	✓		✓	Clean sequence
C5	*C. vancouveriensis*	11	✓			
C6	*C. vancouveriensis*	12	✓		✓	Poor- multiple sequences amplified
CA1	*C. tuberculosum*	13	✓			
CA2	*C. tuberculosum*	14	✓			
CA3	*C. tuberculosum*	15	✓			
CA4	*C. tuberculosum*	16	✓			
CA5	*C. tuberculosum*	17	✓			
CA6	*C. tuberculosum*	18	✓	✓	✓	Poor- multiple sequences
CA7	*C. tuberculosum*	19	✓	✓	✓	Poor- multiple sequences
CA8	*C. tuberculosum*	20	✓			
CA9	*C. tuberculosum*	21	✓			
CA10	*C. tuberculosum*	22	✓			

CA11	*C. tuberculosum*	23	✓	✓	✓	Poor- no sequences
CA12	*C. tuberculosum*	24	✓			
CA13	*C. tuberculosum*	25	✓			
CA14	*C. tuberculosum*	26	✓			
63	*B. plumosa*	27	✓		✓	
64	*C. vancouveriensis*	28	✓		✓	

Table 2. PCR and sequence results from tank experiment.

ID #	Treatment	UPA?	ITS?	*A. marginata* ITS?	Sequenced?	Result if sequenced?
A1	Algae	✓				
A2	Algae	✓				
A3	Algae	✓				
A4	Algae	✓				
A5	Algae	✓				
AS1	Algae + spores	✓				
AS2	Algae + spores	✓		✓	✓	*A. marginata*
AS3	Algae + spores	✓	✓	✓	✓	*A. marginata*
AS4	Algae + spores		✓	✓	✓	*A. marginata*
AS5	Algae + spores		✓	✓	✓	*A. marginata*
XS1	Autoclaved algae + spores	✓	✓	✓	✓	*A. marginata*
XS2	Autoclaved algae + spores		✓			
XS3	Autoclaved algae + spores	✓	✓			
XS4	Autoclaved algae + spores	✓	✓			
XS5	Autoclaved algae + spores	✓	✓			

Fig. 1. Landsat imagery depicting geographic location of the San Juan Islands within the Salish Sea region. Cattle Point, where our samples were collected, is shown at the southeastern most tip of San Juan Island.

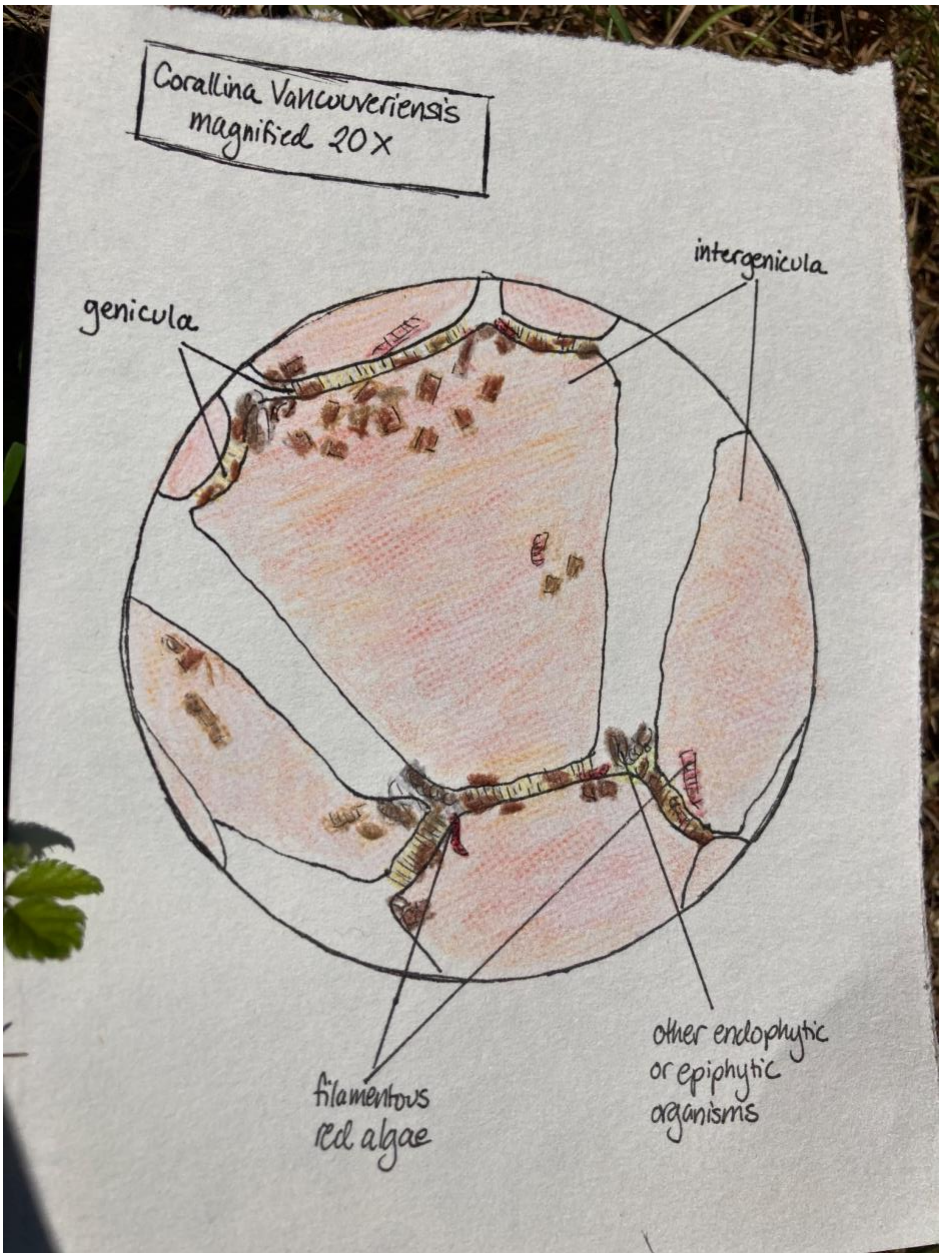


Fig. 2. Drawing of *C. vancouveriensis* specimen at 20X magnification. Shows microscopic organisms inhabiting the genicula and intergenicula of coralline algae, including red algae and potential kelp gametophytes.

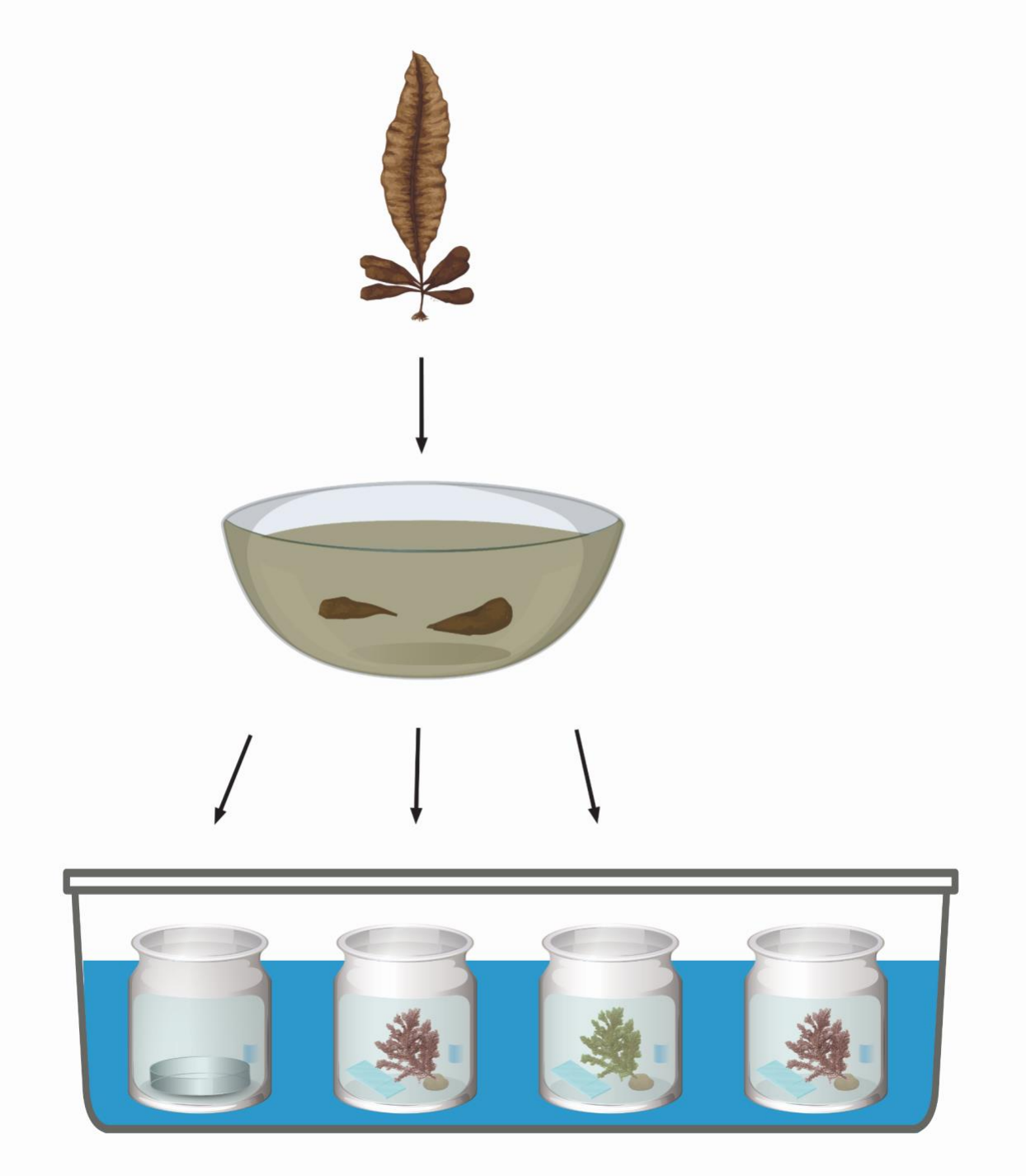


Fig. 3. Experimental setup of tank experiment. *Alaria marginata* reproductive thalli were collected from dock, stressed overnight (see Materials & Methods, Tank Experiment), and the released spores were added to one control jar, one jar with live *B. plumosa*, and one jar with

autoclaved *B. plumosa*. *A. marginata* spores were not added to the final jar, which contained only live *B. plumosa*, sterilized seawater, and a settlement plate.

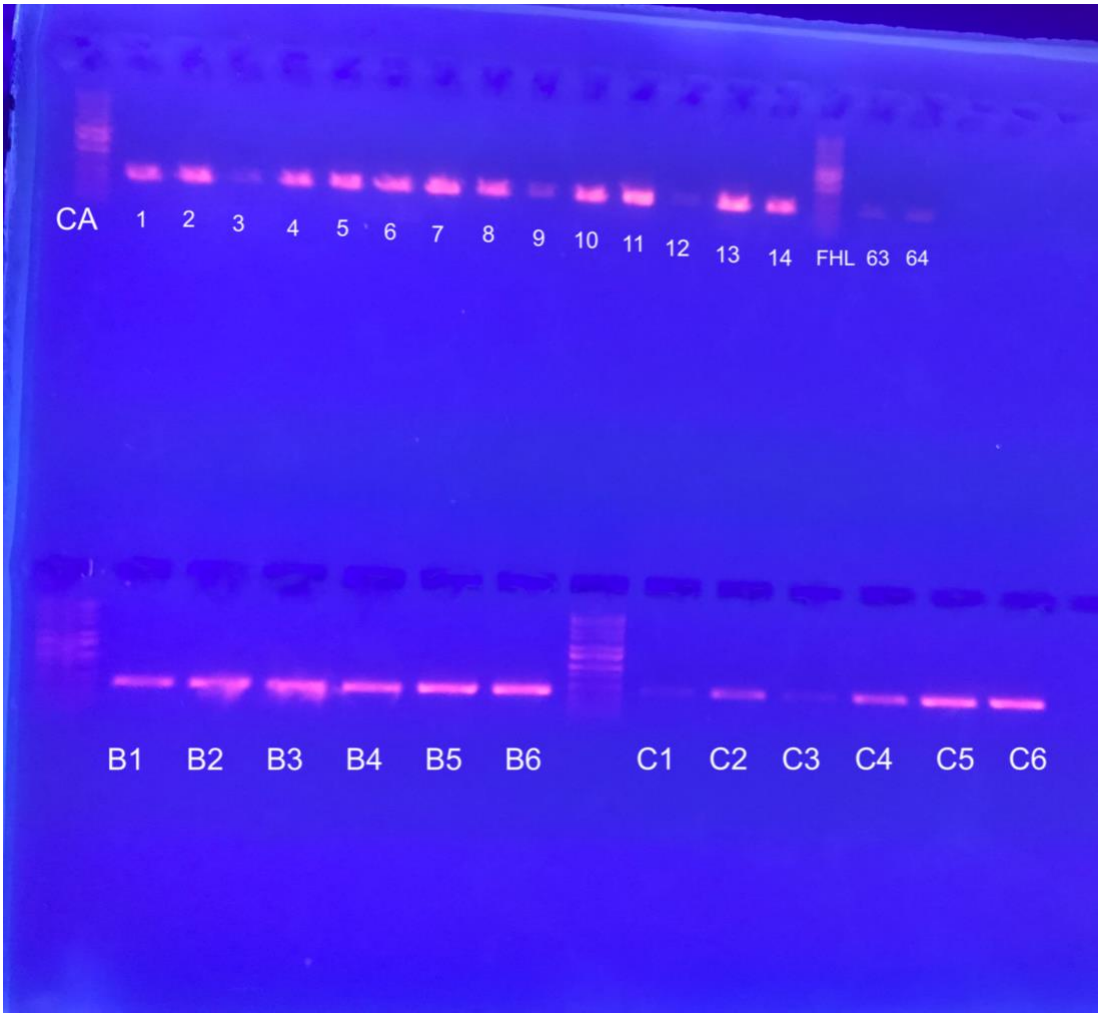


Fig. 4. Gel electrophoresis using PCR product with UPA primers. B# is *B. plumosa*, C# is *C. vancouveriensis*, and CA# is *C. tuberculosum*. FHL63 is *B. plumosa* with no suspected kelp gametophytes, and FHL64 is *C. vancouveriensis* with no suspected kelp gametophytes.

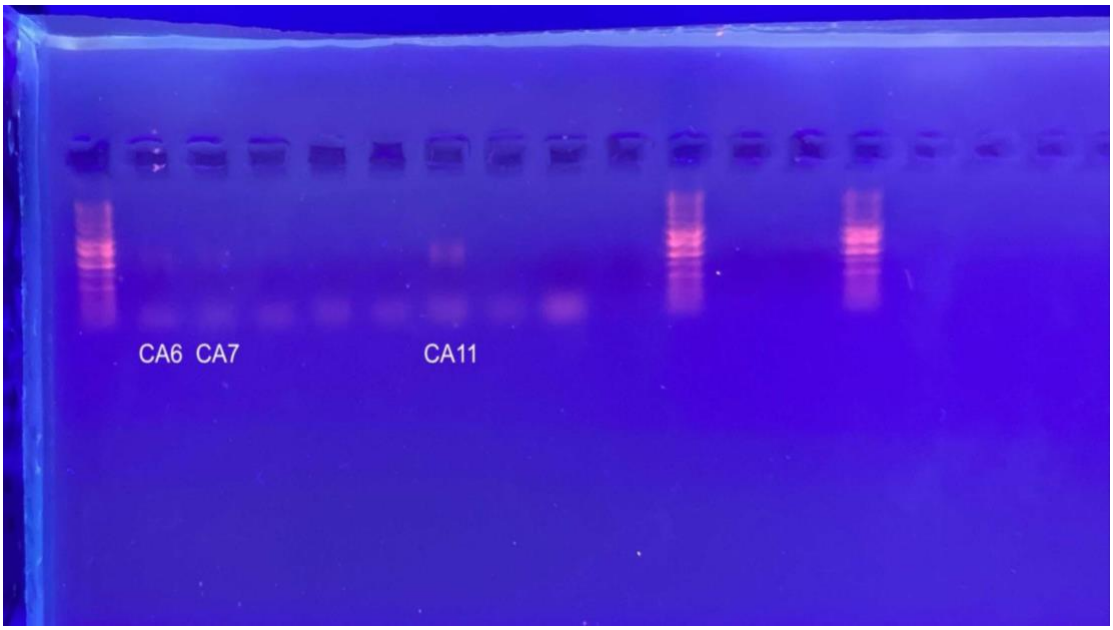


Fig. 5. Gel electrophoresis using PCR product with ITS primers. CA# is *C. tuberculosum*.

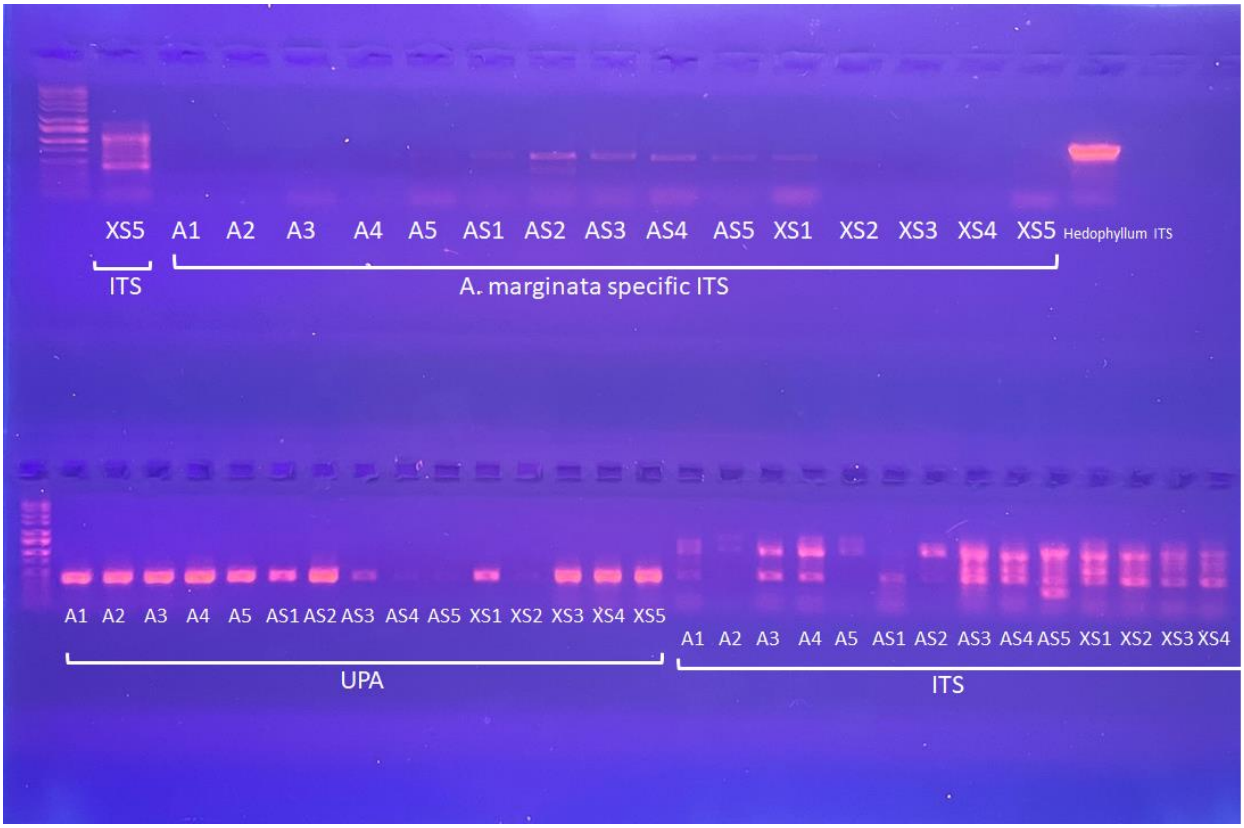


Fig. 6. Tank experiment gel electrophoresis using PCR product with UPA, ITS, and *A. marginata* specific ITS primers. A# is algal substrate, AS# is algal substrate with spores, and AX# is autoclaved algal substrate with spores.

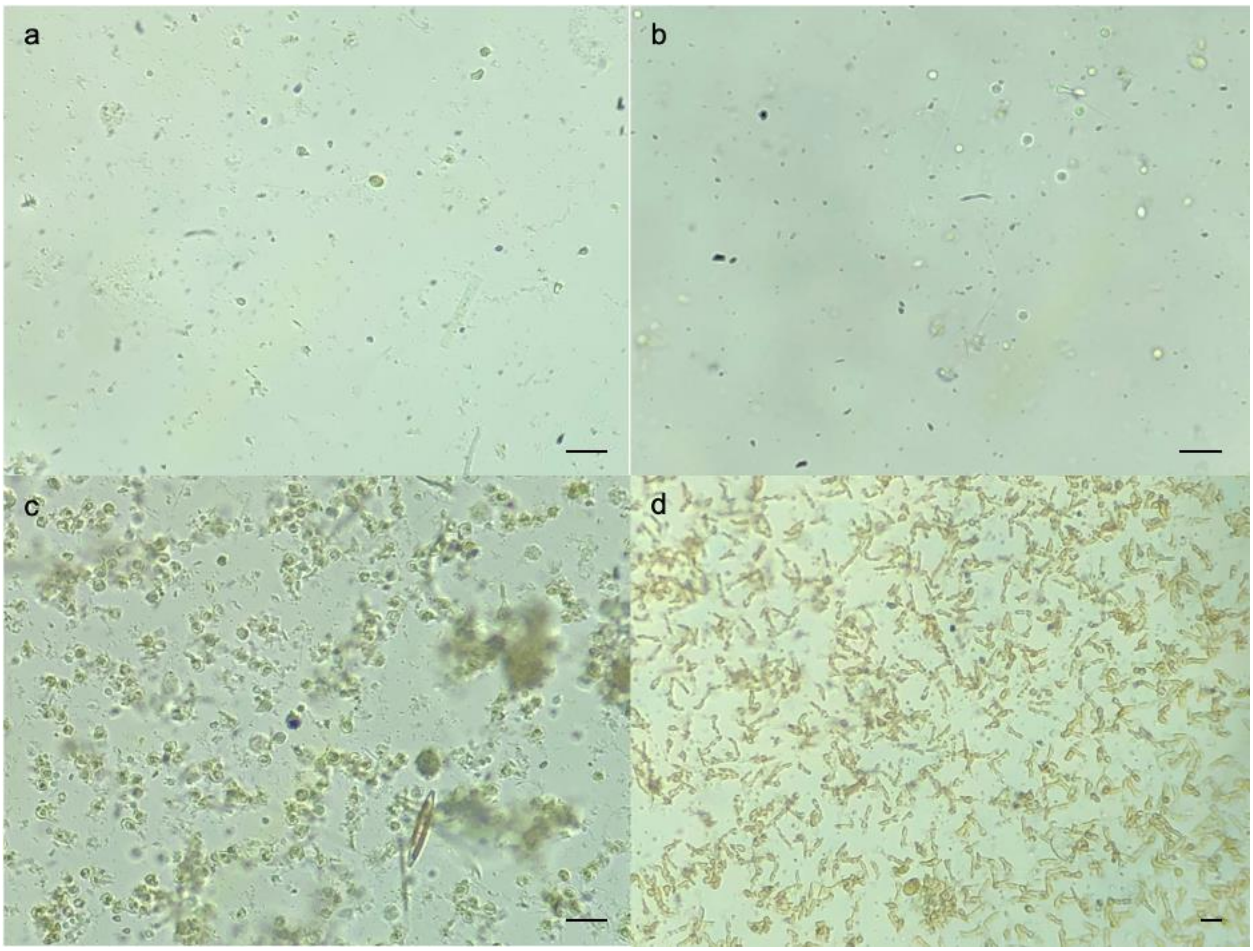
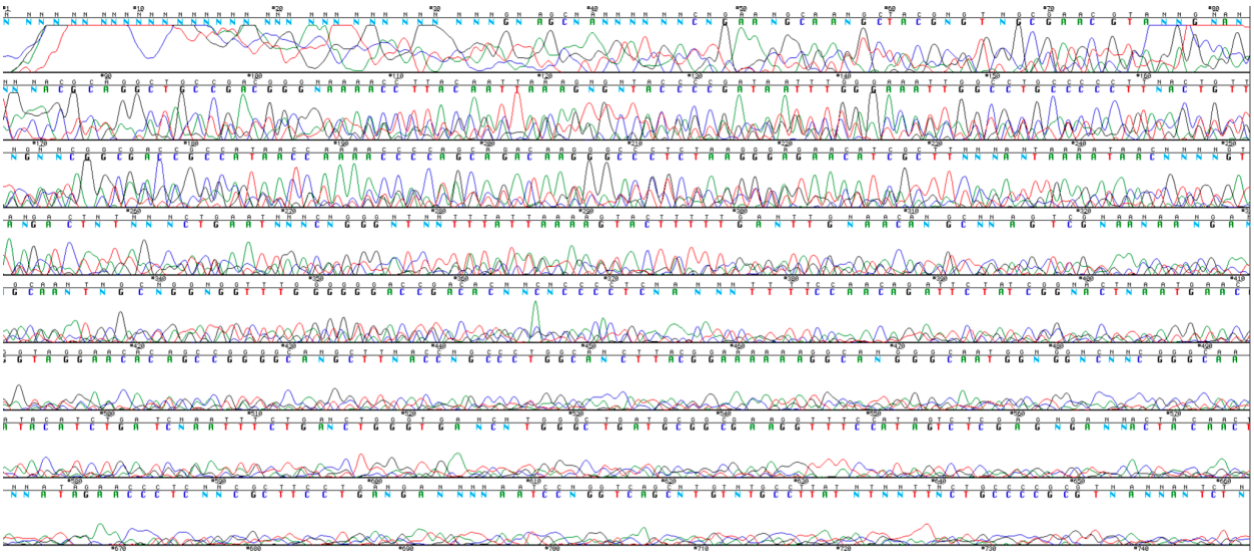
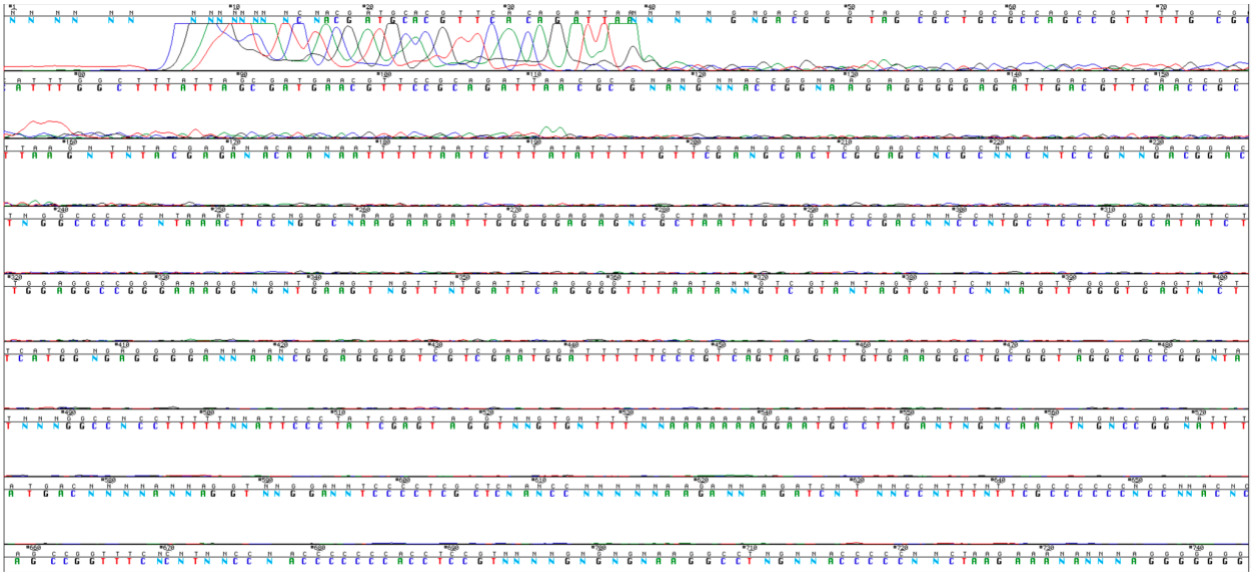


Fig. 7. Settlement plates from the tank experiment. Fig. 7a is the treatment with algae and no spores, b is algae with spores, c is autoclaved algae with spores, and d is no algae with spores. Fig. 7a, b, and c are at 40x magnification, and d is at 20x. The spores in b and c are 3-5 µm in diameter and the scale bar is 20 µm.

Appendix A



Sequence data from CA6 (forward primer P1F strand) with multiple segments of DNA amplified for a single region.



Sequence data from CA11 (reverse primer KG4 strand) with no amplified DNA for the region.