Kelp Gametophyte Culturing and Genetic Analysis Techniques for Conservation and Breeding

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Introduction

- Bull Kelp (*Nereocystis luetkeana*) is the primary canopy-forming species in the coastal environment in the Puget Sound (PS) area of Washington state, providing vital habitat to marine fishes and invertebrates, cycling nutrients, and sequestering carbon.
- Bull kelp is experiencing decreases in population size and local extinction events in the PS due to environmental and anthropogenic stressors.
- Puget Sound Restoration Fund (PSRF) is interested in incorporating both classic phenotype and genotype information in their restoration efforts, particularly in terms of intraspecific hybrids to increase genetic variation; hybridization is desirable because it may result in heterosis; the tendency of a crossbred individual to show qualities superior to those of both parents.
- Bull kelp (*Nereocystis leutkeana*) and giant kelp (*Macrocystis pyrifera*), have been found to hybridize in the wild and have been hybridized in the lab (Lewis and Neushul, 1995).
- Hybrid vigor through intraspecific and interspecific hybridization may aid in kelp populations by introducing novel alleles, resulting in increased genetic diversity, fitness, and resiliency to environmental stressors.

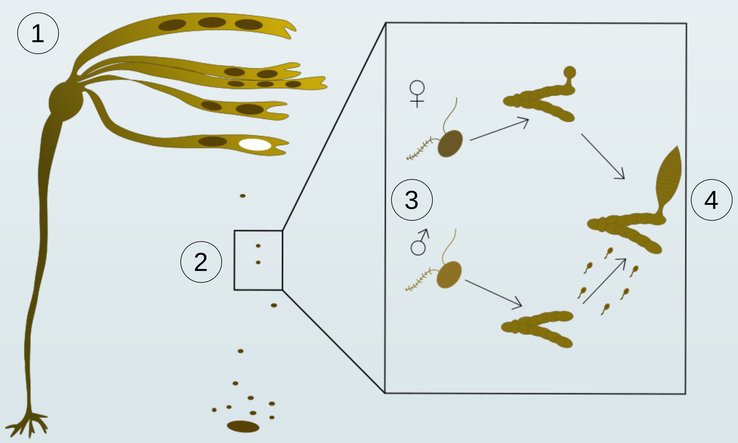


Fig. 1. *Nereocystis luetkeana* life cycle. 1) Adult sporophyte (2N) 2) Zoospores (1N) 3) Gametophytes (1N) 4) Developing sporophyte (2N)

Objectives

- Complete the kelp life cycle in vitro and create a long-term germplasm bank of *Nereocystis* from varying PS population locations that can lead to the recovery of kelp beds in Puget Sound and assist in future research.
- Evaluate the potentials for intra- and interspecific breeding to test for hybrid vigor.
- Molecularly evaluate the success of hybridization between *Macrocystis* and *Nereocystis.*

Methodology

- PSRF sent UWM Alberto Lab 7-20 sporophylls from 20 populations and 7 samples of drifting rafts from within PS, providing the source material to create a long-term germplasm bank; germplasm banks maintain live tissue in culture under dormancy conditions (Fig. 3)
- Gametophytes from three insular PS populations were cultured using standard protocols developed for giant kelp, pooled, and used to inoculate seed string that were outplanted in PS in spring 2020 (Table 1).
- To attempt interspecific hybridization, gametophytes from both species' cultures were fragmented and isolated within 24-well plates. Six variants of crosses were performed of which the sporophytes produced from the treatments were sampled for molecular characterization via direct PCR tests using species specific microsatellite primers and gel electrophoresis.

Location	Code	Total Gametophytes from Location	Total Female Gametophytes	Total Male Gametophytes
Elliott Bay Marina Breakwater	EB	118	62	56
Edmonds	ED	143	75	68
Shilshole Marina Breakwater	SM	80	43	37
Double Bluff	DB	98	47	51
Port Townsend Canal	PT	46	22	24
North Beach	NB	49	25	24
Admiralty Head	AH	69	28	41
Klas Rocks	KR	122	58	64
Possession Point	PP	46	20	26
Hat Island	HI	14	8	6
Lincoln Park	LP	89	45	43
Camano Head	CH	7	3	4
Scatchet Head	SH	130	58	71
Point Vashon	PV	91	44	47
Point Wilson	PW	3	3	0
Day Island	DI	121	60	61
Foulweather Bluff	FB	31	16	15
Salmon Beach	SB	109	51	56

Table 1:) Data table of *Nereocystis* population sample locations, total gametophytes from each location and total of male and female gametophytes isolated.

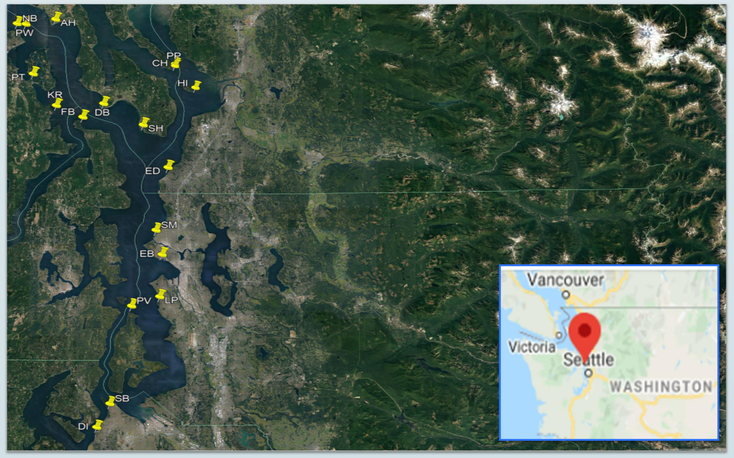
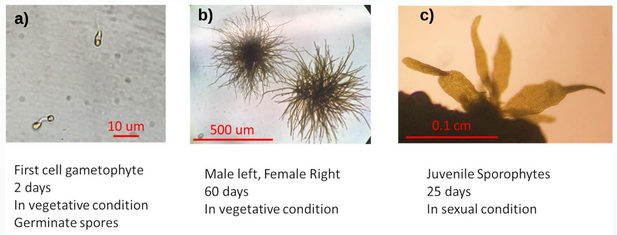


Fig. 2:) Map of *Nereocystis* sample collection sites in the Washington Puget Sound area.

Fig. 3) Process from Spore to Sporophyte: a) Spore germination. **b)** Gametophyte development in lab culture. c) Juvenile sporophyte from gametophytes



Results

Efforts to create *Nereocystis* long term germplasm bank were successful with a total of 1,402 gametophytes preserved. Cultivation of *Nereocystis* gametophytes to sexual maturity with standard *Macrocystis* protocol had low success rates, suggesting culturing conditions for *Nereocystis* have differing parameters compared to *Macrocystis* cultivation.

Our previous molecular results proved inconclusive results of definitive hybridization by genetically analyzing the sporophytes produced. Through our observations of interspecific hybridization, there was expression of sex-specific limitations to hybridizing bull and giant kelp. From the six interspecific crosses conducted, only the ♀*Macrocystis* x ♂*Nereocystis* treatment produced sporophytes. These sporophytes however still did not express hybridization in the molecular tests.

Discussion

Without amplification of both *Macrocystis* and *Nereocystis* species-specific promoter regions of the tested microsatellite regions, we are unable to support that the sporophytes produced from the ♀*Macrocystis* x ♂*Nereocystis* treatment were true interspecific hybrids. Thus, further work is needed.

Low survival and sporophyte production suggested that ideal conditions for maintenance, culture, and crossing of bull kelp gametophytes are unique from giant kelp and warrants additional study.

Further Study

- Future work could include integrating populations from different co-ancestry groups (i.e. greater genetic variability).
- Continued conservation and restoration efforts to test the viability of hybridization for conservation and restoration of bull kelp will protect the integrity coastal environments in PS, including aquaculture, fisheries, and the survival of native marine mammals.
- Using gametophytes of known parentage and genotype allows future genome wide-associated studies to be conducted, from which genetic drivers of desirable traits (e.g., growth rate, temperature and stress tolerance, fecundity) can be identified and optimized to ensure persistence of bull kelp.

Acknowledgements

We would like to thank Dr. Filipe Aos Alberto and Dr. Rachael M Wade for mentorship and advice for the project, We also thank collaborators at UCSB and PSRF staff for collections and outplanting.

References

Lewis, R. J., & Neushul, M. (1995).Intergeneric hybridization among five genera of the family Lessoniaceae (Phaeophyceae) and evidence for polyploidy in a fertile *PelaeophycusxMacrocystis* hybrid.. *Journal of Phycology*, *31*(6), 1012–1017. doi: 10.1111/j.0022-3646.1995.01012.x