Testing Nutrient Media for Kelp Gametophyte Growth

Brandon Wolfson, Elizabeth Roux, Michael Marty-Rivera, M.S.
Kelp Ark, 2451 Signal St, San Pedro, CA 90731

Background

Kelp forests are some of the most productive marine ecosystems, giving critical support to coastal communities, and ocean habitats. These ecosystems are currently threatened by climate change, pollution, and habitat destruction.

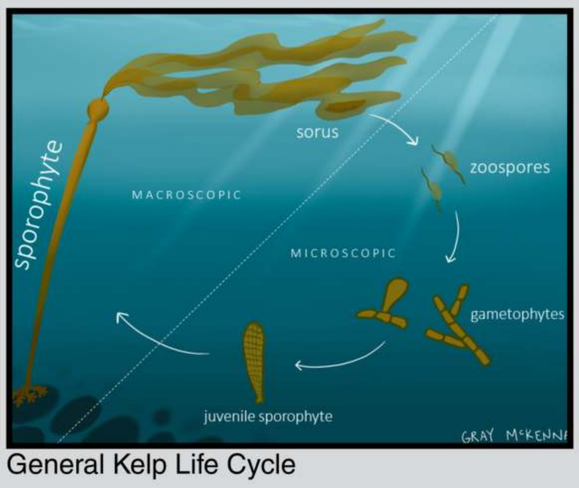
Our study will focus on testing three different nutrient growth medias to measure growth rate in kelp species. We will be working on three kelp species found in Southern California which are of ecological and economical importance.

Traditionally, kelp laboratory cultivation has used PES (Provasoli's Enriched Seawater) medium which contains glycerophosphate, a sugar-phosphate compound that may select for the growth of heterotrophic microbes. This change in the kelp environment over nutrients alters the microbial community composition, and may have consequences on the natural development of kelp.

Macrocystis Pyrifera (MP)

Eisenia Arborea (EA)

Egregia Menziesii (EM)



General Kelp Life Cycle

Materials & Methods

Three kelp species were studied:

Macrocystis pyrifera (MP)
Eisenia arborea (EA)
Egregia menziesii (EM)
2 isolated male and 2 female gametophytes for each species.

Three nutrient media were tested:

- L1: Enriched seawater medium with N:P ratio of 16:1
- F/2: Marine algae medium using Tris buffer, trace metals, and vitamins (pH 8.0-8.2)
- PES: F/2-derived medium with added trace elements and glycerophosphate

Gametophytes were cultured in 3 separate 24-well plates, each containing different media, with weekly microscopy imaging over 6 weeks. Each plate having undergone media change once every 2 weeks.

Area measurements were made using Fiji image analysis software. Growth rates were calculated using specific growth rate formula: $\mu = (\ln(X_2) - \ln(X_1)) / (t_2 - t_1)$, where X represents measured area and t represents time.

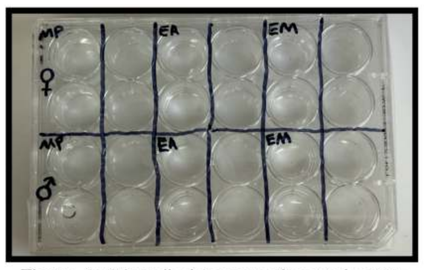


Figure 1: 24-well plate experimental setup used for isolation and culturing kelp gametophytes in different nutrient media.

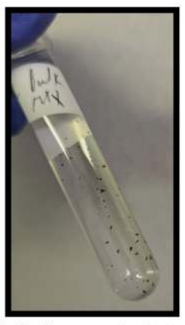


Figure 2: Bulk gametophyte mix for reference of size with the naked eye

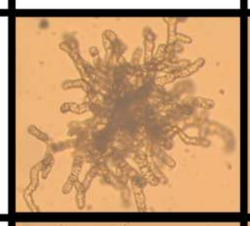
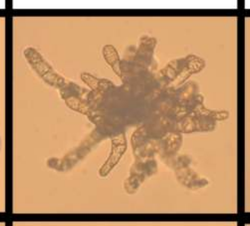
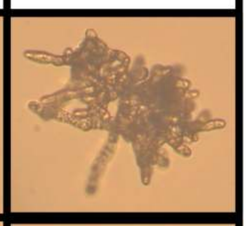
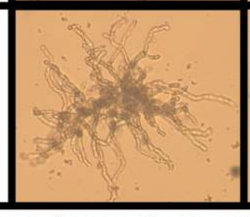
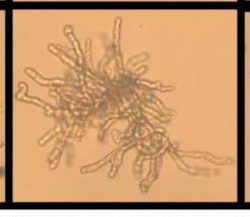
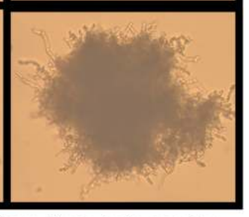
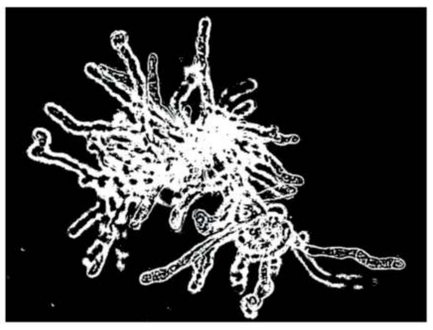
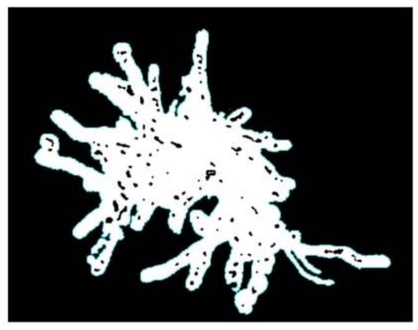
	MP	EA	EM
Female Gamete			
Male Gamete			

Table 1: Microscopy images of male and female gametophytes from three kelp species -- Macrocystis pyrifera (MP), Eisenia arborea (EA), and Egregia menziesii (EM) -- grown under experimental nutrient treatments. Gametophytes display sexual dimorphism present by cell size and thickness.

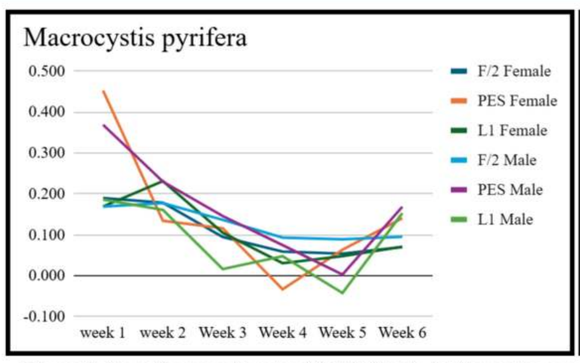


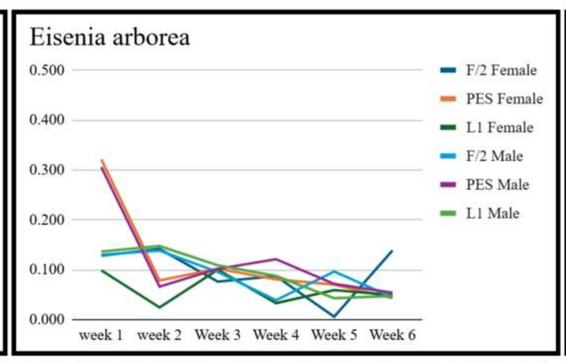


Converted into Binary for Analysis ⟶ Processing for Greater Accuracy

Final Analyzed Particle using Fiji Analysis:
Area = **122,213 µm^2**

Results





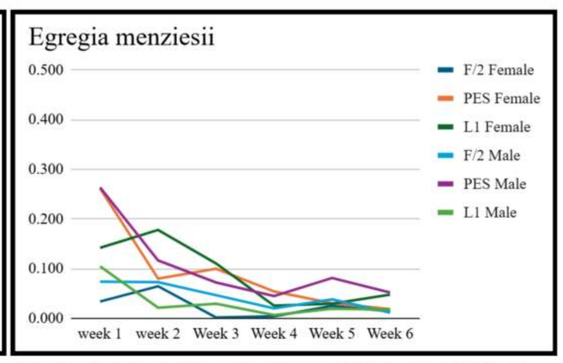


Figure 3: Specific growth rates (SGR) (Units in %growth per week) of Macrocystis pyrifera, Eisenia arborea, and Egregia menziesii male and female gametophytes grown in three different media (PES, F/2, and L1) over six weeks. Growth rates (µ) were calculated using the equation $\mu = (\ln(X_2) - \ln(X_1)) / (t_2 - t_1)$, where X is gametophyte area in µm². Negative values indicate a decrease in size between time points.

The preliminary results indicated that species-specific and sex-specific responses occurred for different nutrient media:

- Macrocystis pyrifera: PES Female and PES Male were initially the fastest growing treatments and declined rapidly before recovering partially towards week 6. F/2 and L1 treatment had moderate initial growth and suffered steady declines.
- Eisenia arborea: PES Female peaked at week 1 but collapsed quickly, while the other treatments all had lower, flat tendancies after week 2. F/2 and L1 treatment were relatively consistent.
- Egregia menziesii: PES Male and Female were initially the fastest growing, but continued decline steadily. F/2 Female was low the entire study, and all treatments had modest changes in growth compared to the other species.

Discussion

- The extreme early growth of M. pyrifera and its later decline suggest a growth strategy for this species to take advantage of nutrient-rich upwelling conditions by requiring strong early growth.
- The relatively consistent and significant growth of E. arborea across all media for both sexes suggests a mid-water ecological niche will select for flexible gametophytes who can thrive in varied nutrient conditions. Thus, E. arborea may be ideal for typical or standardized cultivation standards.
- There is likely high variation in growth because of E. menziesii's particular intertidal habitat where it is subjected to highly variable habitat conditions, thus favoring a more physiologically flexible gametophyte. E. menziesii may require specific micronutrient ratios that are not present in common media.
- Shortcomings during this experiment involve systematic human error from flaws in data collection protocol and gametophyte imaging
- These findings have implications for kelp aquaculture, suggesting that the differing responses of the species indicate that if cultivation technologies and best management practices are to be used for restoration efforts, these protocols should be based on species-specific conditions, rather than a set of standardized cultivation protocols to understand the most optimal kelp growth and production.

Conclusion

- The results of this research indicate that nutrient media composition is fundamentally important for the growth of kelp gametophytes at early life stages, with varying species and sex responses. As such, these results challenge the idea that one particular medium (PES) is suitable for all kelp cultivation purposes.
- This research could help push down cost of kelp farming, PES is expensive both made and premade, L1 and F/2 is a lot cheaper, supporting cheaper means of production while keeping efficient nutrient content.
- These results reinforce the importance of developing individualized growth requirements for kelp along species-based and sex-based differences.
- Further research should be conducted to study the microbial community surrounding gametophytes grown in each of these three different media to see if the sugar-containing compounds that help in the successful culture establishment and growth, play a role in these different media.
- Understanding the requirements of early life stages is critical for further developments of kelp cultivation techniques, promoting sustainable development of aquaculture, and improving conservation efforts to protect vital marine ecosystems.

References

Redmond, S., L. Green, C. Yarish, , J. Kim, and C. Neefus. 2014. New England Seaweed Culture Handbook-Nursery Systems. Connecticut Sea Grant CTSG-14-01. 92 pp.
"Fiji: ImageJ." Fiji, https://fiji.sc/.
Andersen, R. A., editor. Algal Culturing Techniques. Academic Press, 21 Jan. 2005.

Acknowledgments

We thank our mentor Michael Marty-Rivera, M.S., for guidance throughout this research project.
We acknowledge KelpArk and AltaSea for providing internship opportunities and laboratory resources.
Special thanks to Emily Aguirre, Ph.D., for providing the project concept idea.
B. Wolfson is supported by the National Science Foundation grant #2413050.