

Kelp, *Saccharina* spp, population genetics in New England, US, for guiding a breeding program of thermally resilient strains

Simona AUGYTE^{*1}, Jean-Luc JANNINK^{*2}, Xiaowei MAO^{*2, 3}, Mao HUANG^{*2},
Kelly ROBBINS^{*2}, Matt HARE^{*4}, Schery UMANZOR^{*1}, Michael MARTY-RIVERA^{*1},
Yaoguang LI^{*1}, Scott LINDELL^{*5}, David BAILEY^{*5}, Charles YARISH^{*1}

Abstract: The cold-water sugar kelp, *Saccharina latissima* has a circumboreal distribution and in the Northwest Atlantic is at its southern distributional limits in Long Island Sound. An understanding of genetic diversity of natural kelp populations is critical for making recommendations for breeding and cultivation efforts of the growing seaweed aquaculture sector in the US. An important component of the ARPA-E's MARINER project is selectively breeding *Saccharina* spp. in order to improve overall productivity for biofuels, feeds and food.

Historical records indicate the presence of regional kelp ecotypes based on physiological tolerance, specifically temperature. We made collections of 15 wild *Saccharina* spp. populations via SCUBA along the New England coast. Microscopic gametophytes were isolated and the parental populations were used to make over 500 hybrid crosses that were planted at several farm locations over several years. We then used genome-wide single nucleotide polymorphism data to explore the genetic structure of the kelp throughout this region. An assessment of the sequence diversity revealed distinct genetic variation between the Gulf of Maine and Southern New England ($F_{ST} > 0.25$), confirming that Cape Cod acts as a barrier to *S. latissima* gene flow. Furthermore, based on the analysis of molecular variance (AMOVA), we found the largest variance (58%) within sites. We also observed admixture among three ancestral populations and isolation by distance. Future steps for this project include skim sequencing the haploid microscopic gametophytes to identify trait heritability, phenotypic diversity observed for both morphological traits and tissue composition, and genomic selection. Furthermore, in the future, we plan to place our sequence data into a larger context to include samples from sites in the east Atlantic and Pacific Oceans.

Key words: *Saccharina*, population genetics, breeding, thermal resilience

Introduction

For centuries, humans have harvested different seaweeds for food, medicinal purposes, feed, and more recently as raw materials for industrial processes (Augyte *et al.*, 2018; Grebe *et al.*, 2019; Kim, *et al.*, 2019). Over the years, with human population rise, seaweeds have become increasingly

important in global food security. Worldwide, seaweed aquaculture is an important component of total marine aquaculture production, and is experiencing exponential growth in the last 50 years (Kim *et al.*, 2014; Augyte *et al.*, 2017; Langdon *et al.*, 2019; FAO, 2020). Compared to Asian countries including China, Korea, Japan, Indonesia and the Philippines, seaweed aquaculture is a young

2020年12月11日受理 (Accepted on December 11, 2020)

^{*1} Dept. of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT 06901, USA

^{*2} Oregon State University, Fisheries and Wildlife Dept., Hatfield Marine Science Center, Newport, OR 97365, USA

^{*3} Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China

^{*4} Department of Natural Resources and the Environment, Cornell University, Ithaca, NY 14853, USA

^{*5} Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole MA 02543, USA
E-mail: simona.augyte "at" uconn.edu

industry in the United States of America (Kim *et al.*, 2019). It is estimated that the U.S. Farmed seaweed production in the year 2019 was 249,000 – 272,000 kg wet weight with 80% of this production coming from *Saccharina latissima* or sugar kelp (Piconi *et al.*, 2020). It is estimated that edible seaweed production will double in the next five years and will increase by four times by 2035 (Piconi *et al.*, 2020).

Various studies have addressed the gametophyte growth, propagation, and thermal tolerance, as well as ecosystem benefits of seaweed cultivation in the open – ocean (Egan and Yarish, 1988; Kim *et al.*, 2014; Augyte *et al.*, 2017; 2018; Wade *et al.*, 2020), however, a lot remains to be done to achieve a deep understanding of kelp ecophysiology and application to cultivation. To meet the growing demand for increased seaweed aquaculture production, the MARINER program funded by the U.S. Dept. of Energy is focused on increasing production for biofuels, animal feed and human food. As interest in commercially viable species continues, it is therefore critical to have baseline information regarding the underlying genetics of the wild populations to guide sustainable practices. Furthermore, an important component of breeding superior cultivars is selecting kelp strains that are high yielding, and disease and thermally tolerant. The purpose of this study was to assess the genetic structure of wild *S. latissima* populations in Northwest Atlantic, from the southern range of its distribution, throughout Southern New England to the Gulf of Maine. The results of this study will inform managers and conservation groups on genetic hotspot areas of diversity, connectivity and gene flow. Finally, this and future research can guide breeding and cultivation efforts of wild *S. latissima* by identifying phenotypic variation and hybrid vigor.

Materials and Methods

Collections of 15 wild *Saccharina* spp. populations via SCUBA along the coast of New England, in the Northwest Atlantic were made. Commercially important morphometric measurements were done to characterize phenotypic variation specifically on blade length, width, and thickness and stipe length and thickness. Tests were run to correlate

environmental variables with morphology. Samples were collected at peak reproduction and sorus material was isolated and meiospores were released to cultivate the microscopic stage of the life cycle, specifically the male and female gametophytes. These gametophytes were used to make over 500 hybrid crosses that were out planted at several aquaculture farms over two years. Parental sporophytic blade material was collected for DNA extraction and were used for genotyping at the Diversity Arrays Technology (DArT) facility in Canberra, Australia. Reduced genomic representation was generated using restriction enzymes and Illumina HiSeq2500 and then reads were processed and single nucleotide polymorphisms (SNPs) were called using proprietary DArT analytical pipelines (Kilian *et al.*, 2012). Genome-wide SNP data coupled with AMOVA (Analysis of Molecular Variance), FST, admixture, isolation by distance and PCoA (Principal Component Analysis) were used to explore the genetic structure of kelp throughout the region.

Results

Large morphological variation was observed across all 15 locations. For example, adult kelp blade lengths ranged from 84.5 cm – 227 cm; blade widths from 3.4 mm – 41.4 mm; blade thickness ranged from 0.8 – 2.28 mm; stipe diameter from 2.17 mm – 14.43 mm; and stipe length from 4.8 cm – 122.7 cm.

The populations within the Gulf of Maine showed significantly higher genetic diversity compared to populations in the southern region, in the Southern New England area. Results further indicate that roughly half (56%) of the total variation exists within locations (AMOVA, p -value<0.001). Isolation by distance, indicated by positive correlation between genetic and geographic distances, was observed for both the Gulf of Maine ($r=0.47$, p -value=0.002) and Long Island Sound ($r=0.94$, p -value=0.125). The pair-wise genomic F_{ST} between the two regions was >0.25 and was supported by the PCoA plots. Despite the isolation by distance pattern in each region, ancestry analyses indicate a complex history of historical gene flow both within and between the regions stemming from three ancestral populations.

Discussion

Historical biogeographic reconstructions reveal that complex kelp originated in the northeast Pacific and diversified over time by colonizing new habitats (Starko *et al.*, 2019). The kelp in the Northwest Atlantic were colonized post-glacially via oceanographic flow through the Arctic (Nielsen *et al.*, 2016; Neiva *et al.*, 2018). In the Atlantic, the kelp migrated south as far as their summer thermal maximum temperature tolerance allowed (Egan and Yarish, 1988). Since the 1980's, this region has experienced significant sea surface warming resulting in northward shifts of isotherms with serious implications for canopy forming seaweeds in both intertidal and subtidal habitats (Wilson *et al.*, 2019). In the Northwest Atlantic, our findings support a major genetic break for *S. latissima* formed by the biogeographic barrier at Cape Cod. Populations north and south of this Cape share some genetic ancestry but have also diverged over time. Despite this deep regional population structure, the genetic variation found within locations accounted for the greatest proportion of the total, indicating abundant local standing diversity available for population adaptation or breeding. Future studies will place this genomic data into a biogeographically larger context. This fundamental genetic data is useful not only for gaining a better understanding of the population structure of *S. latissima*, but also for guiding sustainable seaweed aquaculture. These results can guide management and conservation of kelp ecotypes, specifically by placing efforts to conserve certain genetics and phenotypes.

This study is part of ongoing efforts to selectively breed sugar kelp for large scale food and bioenergy production with increasing focus on germplasm banking to support future cultivation and restoration research (Wade *et al.*, 2020). Hatchery cultivation of microscopic stages of *S. latissima* are on-going and aim to improve the efficiency for selective breeding cultivars that are thermally tolerant and high yielding. Future studies of *S. latissima*, will help identify ecotypes that are best adapted for farming in the off-shore, low nutrient, environment.

Funding was provided by the U.S. Department of Energy, ARPAe MARINER project contract number

DE-AR0000915 and DE-AR0000911. We acknowledge all of the contributions made by M. Chambers and his team at the University of New Hampshire and the volunteers at Wood Hole Oceanographic Institute and the University of Connecticut, Stamford, who helped during the two breeding seasons of this domestication program.

References

- Augyte S., Yarish C., Redmond S., and Kim J. K., 2017: Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services. *J. Appl. Phycol.*, **29**, 1967–1976. (doi: 10.1007/s10811-017-1102-x)
- Augyte S., Umanzor S., Yarish C., and Lindell S., 2018: Enhancing marine ecosystem services via kelp aquaculture. *ISAP newsletter*, **December**, 10–14.
- Egan B., and Yarish C., 1988: The distribution of the genus *Laminaria* (Phaeophyta) at its southern limit in the Western Atlantic Ocean. *Botanica Marina*, **31**, 155–161.
- FAO, 2020: The State of World Fisheries and Aquaculture 2020. Sustainability in action, FAO, Rome, 244pp. (<https://doi.org/10.4060/ca9229en>)
- Grebe, G. S., Byron C. J., St. Gelais A., Kotowicz D. M., and Olson T. K., 2019: An ecosystem approach to kelp aquaculture in the Americas and Europe. *Aquac. Rep.*, **15**, 100215. (doi: 10.1016/j.aqrep.2019.100215)
- Kilian A., Wenzl P., Huttner E., Carling J., Xia L., Blois H., Caig V., Heller-Uszynska K., Jaccoud D., Hopper C., Aschenbrenner-Kilian M., Evers M., Peng K., Cayla C., Hok P., and Uszynski G., 2012: Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms, in “Data Production and Analysis in Population Genomics, Methods and Protocols” (ed. by Pompanon F., and Bonin A.), *Methods in Molecular Biology*, vol 888, Humana Press, Totowa, NJ, pp.67–89. (https://doi.org/10.1007/978-1-61779-870-2_5)
- Kim J. K., Kraemer G. P., and Yarish C., 2014: Field scale evaluation of seaweed aquaculture

- as a nutrient bioextraction strategy in Long Island Sound and the Bronx River Estuary. *Aquaculture*, 433, 148–156. (doi: 10.1016/j.aquaculture.2014.05.034)
- Kim J. K., Stekoll M., and Yarish C., 2019: Opportunities, challenges and future directions of open-water seaweed aquaculture in the United States. *Phycologia*, **58(5)**, 446–461. (doi: 10.1080/00318884.2019.1625611)
- Langton R., Augyte S., Price N., Forster J., Noji T., Grebe G., St. Gelais A., and Byron C. J., 2019: An Ecosystem Approach to the Culture of Seaweed. NOAA Tech. Memo. NMFS-F/SPO-195, 24 pp. (<https://spo.nmfs.noaa.gov/content/tech-memo/ecosystem-approach-culture-seaweed>)
- Nielsen M. M., Paulino C., Neiva J., Krause-Jensen D., Bruhn A., and Serrão E. A., 2016: Genetic diversity of *Saccharina latissima* (Phaeophyceae) along a salinity gradient in the North Sea-Baltic Sea transition zone. *J. Phycol.*, **52(4)**, 523–531. (doi: 10.1111/jpy.12428)
- Neiva J., Paulino C., Nielsen M. M., Krause-Jensen D., Saunders G. W., Assis J., Bárbara I., Tamigneaux É., Gouveia L., Aires T., Marbà N., Bruhn A., Pearson G. A., and Serrão E. A., 2018: Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissimi*. *Sci. Rep.*, **8**, 1112. (doi: 10.1038/s41598-018-19620-7)
- Piconi P., Veidenheimer R., and Chase B., 2020: Edible Seaweed Market Analysis. Island Institute, Rockland, ME, 56pp. (<http://www.islandinstitute.org/edible-seaweed-market-analysis>)
- Starko S., Soto Gomez M., Darby H., Demes K. W., Kawai H., Yotsukura N., Lindstrom S. C., Keeling P. J., Graham S. W., and Martone P. T., 2019: A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem. *Mol. Phylogenet. Evol.*, **136**, 138–150. (doi: 10.1016/j.ympev.2019.04.012)
- Wade R., Augyte S., Harden M., Nuzhdin S., Yarish C., and Alberto F., 2020: Macroalgal germplasm banking for conservation, food security, and industry. *PLoS Biol.*, **18(2)**, e3000641. (doi: 10.1371/journal.pbio.3000641)
- Wilson K. L., Skinner M. A., and Lotze H. K., 2019: Projected 21st-century distribution of canopy-forming seaweeds in the Northwest Atlantic with climate change. *Divers. Distrib.*, **25**, 582–602. (doi: 10.1111/ddi.12897)

Annotated Bibliography of Key references

(1) Langton R., Augyte S., Price N., Forster J., Noji T., Grebe G., St. Gelais A., and Byron C. J., 2019: An Ecosystem Approach to the Culture of Seaweed. NOAA Tech. Memo. NMFS-F/SPO-195, 24pp. (<https://spo.nmfs.noaa.gov/content/tech-memo/ecosystem-approach-culture-seaweed>)

Seaweeds are a significant component of current marine aquaculture production and will play an increasing role in global food security as the human population increases rapidly over the next 30 years. Seaweed farming is analogous to plant based agriculture except that the crop is cultured in a marine environment. It differs from agriculture in that seaweeds do not require tillable land, fertilization or freshwater, which are resources that may ultimately constrain the expansion of agriculture. Seaweeds are converted into a variety of goods, such as food and nutritional supplements for humans and livestock, fertilizer, unique biochemical and biofuels. Wild and cultured seaweed also offer multiple ecosystem services, such as bioremediation for coastal pollution, localized control of ocean acidification, mitigation of climate change and habitat for other marine organisms. Incorporation of seaweeds into marine aquaculture farms in the United States (U.S.) is, however, not without its challenges. Seaweed is an unconventional food which necessitates establishing product acceptability, creating a sustained market and then balancing demand with a consistent supply for long term economic profitability. Seaweed farms also need to be developed in a manner that is compatible with wild capture fisheries, marine mammal migrations and other users of the marine environment. A comprehensive understanding of the role that cultured seaweeds play in the marine ecosystem is necessary in order to determine not only the economic value of the goods produced but also the ecosystem services offered by marine farming activities. This will result in a better understanding

of how an ecosystem approach to aquaculture incorporates the role and need for both the goods and services these macroalgae will provide.

(2) Augyte S., Umanzor S., Yarish C., and Lindell S., 2018: Enhancing marine ecosystem services via kelp aquaculture. *ISAP newsletter*, December, 10–14. (https://docs.wixstatic.com/ugd/e9f50b_f3edc9d188f248a18061e09cfa25b5d9.pdf)

For centuries, humans have harvested different seaweeds for food, medicinal purposes, feed, and more recently as raw materials for industrial processes. Currently, wild harvested seaweed account for less than 5% of the total worldwide supply (FAO, 2018). The majority of seaweed production is provided via aquaculture, with 99% of the production taking place in Asia including China, Korea, Japan, Indonesia, and the Philippines, worth US \$11.7 billion annually (FAO, 2018). Although seaweed aquaculture is a fast expanding industry, the global demand for seaweed-based products is surpassing the supply. Such demands necessitate either domesticating new species or further expanding the productivity of the existing leading seaweeds. According to the FAO, only a few species dominate seaweed farming, including two brown kelps, *Saccharina japonica* and *Undaria pinnatifida* (Buschmann *et al.*, 2018). To meet the present market requirements and to contribute in reducing the over-exploitation of wild stocks, experimental trials worldwide have assessed the performance of newly farmed seaweeds as potential products, particularly kelp species that have been traditionally harvested from wild populations.

(3) Augyte S., Yarish C., and Neefus C. D., 2019: Thermal and light impacts on the early growth stages of the kelp *Saccharina angustissima*

(Laminariales, Phaeophyceae). *Algae*, **34**, 153–162. (doi.org/10.4490/algae.2019.34.5.12)

Anthropogenic disturbances, including coastal habitat modification and climate change are threatening the stability of kelp beds, one of the most diverse and productive marine ecosystems. To test the effect of temperature and irradiance on the microscopic gametophyte and juvenile sporophyte stages of the rare kelp, *Saccharina angustissima*, from Casco Bay, Maine, USA, we carried out two sets of experiments using a temperature gradient table. The first set of experiments combined temperatures between 7–18°C with irradiance at 20, 40, and 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The second set combined temperatures of 3–13°C with irradiance of 10, 100, and 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Over two separate 4-week trials, in 2014 and again in 2015, we monitored gametogenesis, the early growth stages of the gametophytes, and early sporophyte development of this kelp. Gametophytes grew best at temperatures of 8–13°C at the lowest irradiance of 10- $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Light had a significant effect on both male and female gametophyte growth only at the higher temperatures. Temperatures of 8–15°C and irradiance levels of 10–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were conditions for the highest sporophyte growth. Sporophyte and male gametophyte growth was reduced at both temperature extremes—the hottest and coldest temperatures tested. *S. angustissima* is a unique kelp species known only from a very narrow geographic region along the coast of Maine, USA. The coupling of global warming with high light intensity effects might pose stress on the early life-history stages of this kelp, although, as an intertidal species, it could also be better adapted to temperature and light extremes than its subtidal counterpart, *Saccharina latissima*.