Impacts of Cultivation Depth on the Yield and Morphological Characteristics of Three-rib Kelp (*Cymathere triplicata*), Split Kelp (*Hedophyllum nigripes*), and Dragon Kelp (*Eualaria fistulosa*)

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Purpose and Context: Introduction

Commercial seaweed aquaculture in Alaska is presently dominated by sugar kelp (*Saccharina latissima*), with some cultivation of ribbon kelp (*Alaria marginata*), and bull kelp (*Nereocystis luetkeana,* McKinley Research Group 2021). While these species are similar to other kelp species that have been prominent in global industries, their adoption into the Alaskan industry is marked by familiarity rather than opportunity. Alaska is a kelp diversity hotspot, and other species endemic to the region may be important in stabilizing the Alaskan seaweed industry (Stekoll 2019).

We identified three regionally abundant but under-cultivated kelps, three-rib kelp (*Cymathere triplicata*), split kelp (*Hedophyllum nigripes*), and dragon kelp (*Eualaria fistulosa*), to evaluate their performance in farm array conditions. Three-ribbed kelp and dragon kelp are endemic to the northeastern Pacific and are not found elsewhere (Farrugia Drakard et. al, 2023; Li et. al., 2024, Stekoll 2019). Split kelp is a holoarctic species that has not been widely cultivated (Kreissig 2021). Ecologically, three-rib and split kelp are species that function as sub-canopy kelps and can attain high densities in nature, whereas dragon kelp is canopy-forming and potentially more sensitive to hydrodynamics; wild stands of dragon kelp typically occur at lower adult densities than cosmopolitan sub-canopy species (Lees et. al. 2025). These traits suggest that cultivation outcomes may be density-dependent and species-specific.

Marine farmers have limited ability to control the factors that may influence the yield and phenotype of cultivated macroalgae. In Alaska, regulations prohibit the addition of artificial nutrients, so farmers must rely on natural patterns of nutrient availability and distribution (State of Alaska, Aquatic Farming Regulations and Statutes). Likewise, ocean currents, tidal flow, and light conditions cannot be manipulated beyond the initial site selection, an aspect often determined more by proximity to ports or infrastructure than by conditions ideal for kelp growth (Visch et. al, 2020; Boderskovet et. al, 2021). As a result, farmers primarily manage structural aspects of their farming systems, such as the spacing between cultivation lines, the depth of those lines below the surface, and, to some extent, the density of sporophytes (kelp plants) along each line (Stekoll et. al, 2024; Stekoll et. al, 2025).

All three kelp species cultivated in these trials are known to occur naturally from just below the intertidal zone to depths of approximately 30 meters at mean high water (Lees et. al, 2025; Metzger et. al, 2019; Stekoll 2019). Both split kelp and three-ribbed kelp are neutrally buoyant species that on average attain lengths between 0.5-3.0 m making them similar in phenotype and growth habits to sugar kelp (*S. latissima*) a widely cultivated species in the western hemisphere and the most widely cultivated species in Alaska (McKinley Research Group 2021). In contrast, dragon kelp is a floating species that can attain lengths of over 7 m (Stekoll 2019). Thus the majority of the thallus (blade) may be exposed during low tide or as it grows, possibly leading to tissue senescence and loss of material and biomass, as observed in the farming of other floating species such as bull kelp (*N. luetkeana*) when grown at the conventional 2.14 m (7 ft) subsurface (Stekoll et. al, 2024).

In this study, depth was selected as the primary variable of interest because it is relatively simple to control and the three species have been observed naturally growing successfully at different depths (Lees et. al, 2025; Metzger et. al, 2019; Stekoll 2019). Final biomasses and morphometric traits were compared at two depths to test for depth- and species-dependent differences in biomass, frond density, and thallus morphology. We hypothesized that shallower lines would support higher overall growth due to greater light availability, whereas deeper lines would produce thalli with altered morphology under lower irradiance and different hydrodynamic conditions.

This project is conducted in partnership with the Native Village of Ouzinkie and Spruce Island Farms, LLC (Alaska Native owned), within KALI's Alutiiq Grown regional team. Engagement includes employment of an Ouzinkie community farmer to monitor the array at their farm, a hatchery technician trainee from the Alutiiq Grown program, and routine reporting to regional Alaska Native leadership forums to ensure co-production of practice-relevant knowledge.

<u>Activities and Outcomes</u>:

Methods

Seed Production

Seedline of three-rib kelp (*C. triplicata*), split kelp (*H. nigripes*), and dragon kelp (*E. fistulosa*) were established following Flavin et al. 2013 protocols and maintained at the Alaska Ocean Farms Hatchery (AOF, ADF&G Hatchery Permit #2023-5-HA-WE). Seed spools were incubated in the nursery for 6-8 weeks until sporophytes reached 0.5-2 mm. The seed line was tended to daily by the nursery intern hired for the project with oversight from the AOF hatchery manager, Lexa Meyer. Duties for the nursery intern included: assisting with seed kelp collection, cleaning and sanitizing nursery equipment and seed spools, spore release and stocking seed spools, rotating spools to ensure even light coverage, adding nutrients, performing water changes, and verifying tank circulation system integrity and checking for leaks.

It was challenging to find all three species in a fertile state during mid-summer, when the project planned to create gametophyte cultures for inoculating seed spools. Additionally, the Alaska Department of Fish and Game's Aquatic Farming Division does not currently permit the use of gametophyte cultures across multiple calendar years. These factors influenced the authors' decision to use spore seeding instead of gametophytes to produce the seed line for this experiment, aligning with the seeding methods currently available to kelp farmers in Alaska.

Farm Construction and Deployment

Two identical 75-m experimental arrays were constructed by Alf Pryor of Alaska Ocean Farms (AOF) to evaluate growth of *Cymathaere triplicata*, *Eualaria fistulosa*, and *Hedophyllum nigripes* from seed lines produced at the AOF nursery in fall 2024. Each array comprised six treatment blocks (10 m long × 3.05 m wide). Within each block, two parallel 10-m cultivation lines were installed (one species per block x 2 lines, Figure 1). To test depth effects, three blocks in each array were tensioned to 2.14 m below the surface and three to 3.66 m. These depths were chosen as one corresponds to the conventional farming depth for *S. latissima* (2.14 m) and the other to the average depth that *Eualaria fistulosa* was observed growing near both farms (3.66 m, communication with farmers). One array was deployed and seeded at the Spruce Island farm (Spruce Island Community Farms, Ouzinkie, Alaska) on 14 December 2024; the second was deployed and seeded at the Woody Island farm (AOF, LLC, Kodiak, Alaska) on 19 January 2025. The farms are ~22 mi (35 km) apart within greater Women's Bay on the northeastern side of Kodiak Island. Two 10 m cultivation lines of each species *Cymathaere triplicata*, *Eualaria fistulosa*, and *Hedophyllum nigripe,* were outplanted at each depth (2.41 m and 3.66 m) on each array (4 lines of each species total, per farm, Figure 1).

Cultivation lines were maintained through June 2025, when all project biomass was sampled/harvested and the arrays were removed from the ocean including anchoring systems. Field observations indicate that wild *E. fistulosa* and *H. nigripes* in adjacent beds mature in July–August; however, farm harvests were scheduled earlier (May–June) to capture cultivated peak growth and enable standardized measurements across treatments.

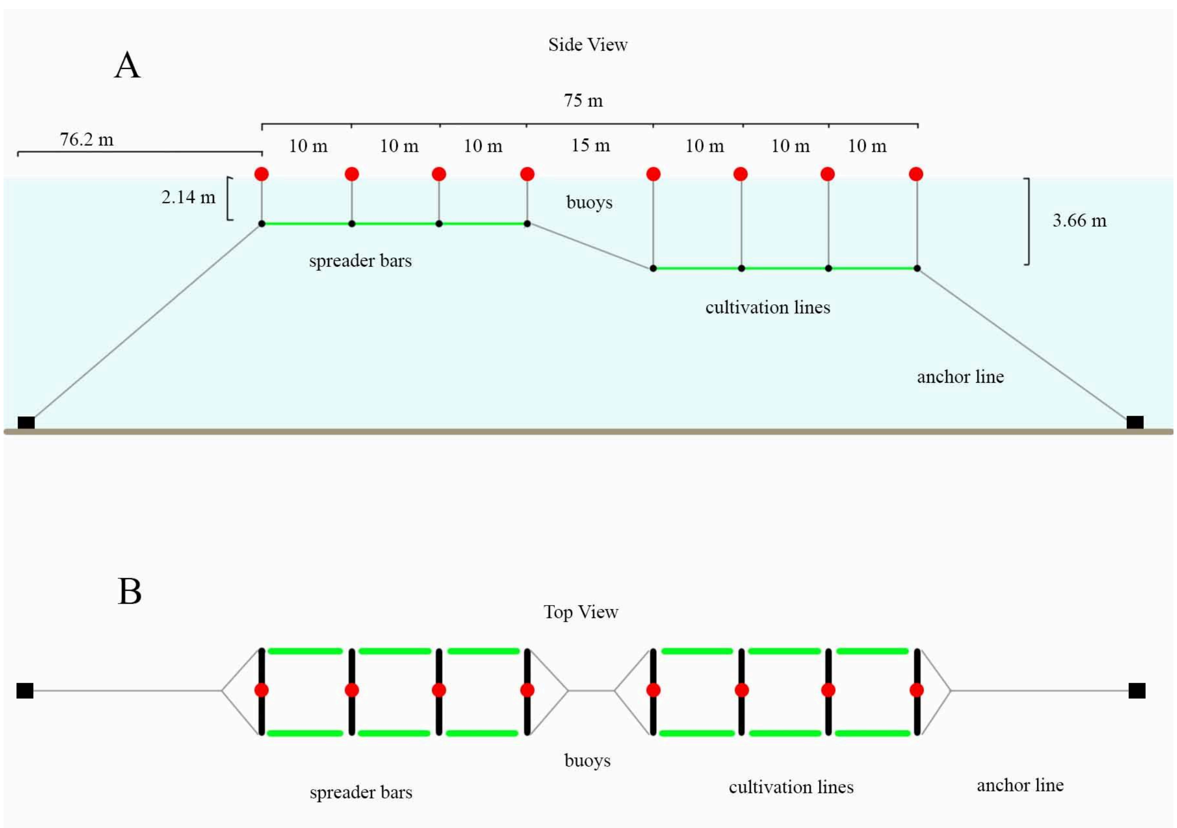

Figure 1. Diagram of the spreader bar arrays constructed by Alaska Ocean Farms, LLC. A presents a side view of the array showing the alteration in depth. B presents a top down view of the array. Black thick lines are the spreader bars, green lines the cultivation lines where the kelp

seed line was outplanted, red circles represent flotation buoys, and the black squares anchors. Two 10 m cultivation lines of each of the three species, *Cymathaere triplicata*, *Eualaria fistulosa*, and *Hedophyllum nigripes,* were outplanted at each of the two depths on each array.

Figure 2. a. Alf Pryor of Alaska Ocean Farms and Duke Delgado of Spruce Island Farms threading the cultivation line through a kelp seed spool prior to outplanting the array near Spruce Island on December 12, 2024; b. Max Lions of the Kodiak Archipelago Leadership Institute and Duke Delgado examine cultivation lines during deployment at the Spruce Island farm; c. the Spruce Island Farm array fully deployed.

Monitoring and Array Maintenance

It was planned that the arrays would be monitored two times per month or after every major storm event from outplanting through harvest. However, lack of access to a vessel prevented Ouzinkie farmers from visiting this site frequently. AOF farmer Alf Pryor was able to check on the Ouzinkie array three times from December through June. He was also able to monitor the AOF Woody Island array with the prescribed frequency.

Harvest and Measurements

At harvest, each 10 m grow line was sampled at 1, 3, 5, 7, and 9 m, effectively sampling half of the biomass (kg · m^{-1}) for each sample. The kelp from the array at Woody Island was harvested on June 18th, 2025 and the kelp on the array at Spruce Island harvested on June 19th, 2025. Sampled biomass was immediately transferred to labelled mesh bags, and their biomass was determined via a scale while on the harvest boat. The samples were transported to the Kodiak Seafood and Marine Science Center for morphometric sampling. If frond densities (frond count · m^{-1}) exceeded 10 for each sample, we haphazardly subsampled 10 individuals to measure frond morphometrics, including frond length (cm), blade length (cm), blade width (cm), blade thickness (mm), and midrib thickness (mm). The mean biomass per frond was estimated by dividing the biomass from each sample by the sample's frond density.

Figure 3. A. Measuring dragon kelp for a subsample from the array at Spruce Island on June 19th, 2025; b. Alf Pryor of Alaska Ocean Farms harvesting kelp from the array at Spruce Island Farm; c. Three-ribbed Kelp (*C. triplicata*) harvested from the array at Spruce Island being measured at the Kodiak Seafood and Marine Science Center on June 20, 2025.

Data Management

All data were recorded in field notebooks (Rite in the Rain) or on printed spreadsheets when sampling ashore. Data were subject to QC/QA prior to analysis. Data analysis was performed in the Statistical package R Studio.

Results

For *Cymathere triplicata*, mean biomass at 2.2 m was 3.672 ± 1.662 kg m^{-1}, with frond density averaging 9.9 ± 4.2 fronds m^{-1}. Blade length averaged 229.3 ± 95.3 cm, blade width 19.9 ± 7.5 cm, and blade thickness 0.25 ± 0.24 mm. Midrib thickness was 1.64 ± 0.59 mm. At 4 m, mean biomass was reduced to 1.748 ± 1.824 kg·m^{-1} with a mean frond density of 8.1 ± 8.7 fronds·m^{-1}. Average blade length was 202.3 ± 109.5 cm, width 17.4 ± 7.5 cm, and thickness 0.20 ± 0.12 mm. Midrib thickness averaged 1.71 ± 0.92 mm.

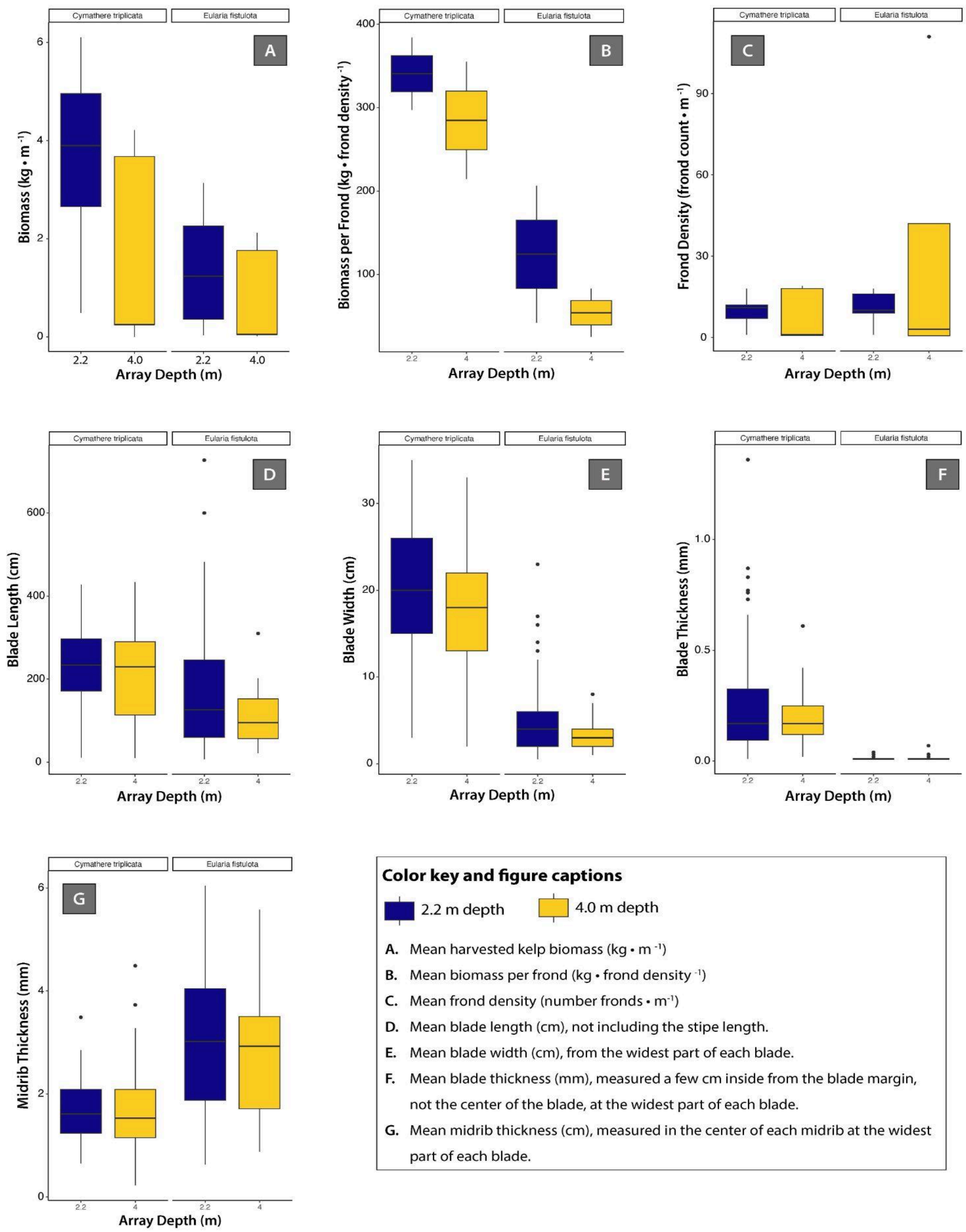

Figure 4. All monitored biomass and biometric data collected for three-rib kelp and dragon kelp (left pair and right pair of boxes in each plot, respectively) at 2.2 m and 4.0 m depth (blue and yellow boxes, respectively). Description for each plot (A-G) is included in the "Color key and figure captions" box in the lower right corner of this figure.

For *Eularia fistulosa*, mean biomass at 2.2 m was 1.293 ± 0.957 kg m^{-1}, with frond density of 11.8 ± 4.4 fronds m^{-1}. Average blade length was 157.9 ± 126.6 cm, width 5.1 ± 3.8 cm, and thickness 0.01 ± 0.01 mm. Midrib thickness was 3.08 ± 1.40 mm. At 4 m, mean biomass was 0.885 ± 0.962 kg m^{-1}, and frond density increased to 34.5 ± 44.5 fronds m^{-1}. Average blade length was 106.4 ± 62.9 cm, width 3.1 ± 1.8 cm, and thickness 0.01 ± 0.01 mm. Midrib thickness was 2.69 ± 1.19 mm.

Although we collected data for the attempt with *Hedophyllum nigripes*, the biomass was sparse and diminutive, and it was unclear whether the species on the line was, in fact, *Hedophyllum* or whether the biomass present on the grow lines was from wild-set or nursery-cultivated seed. Therefore, we did not include these data in the analysis for this work.

Challenges and Lessons Learned: Discussion

This study provides the first farm-based evaluation of three-rib kelp (*C. triplicata*) and dragon kelp (*E. fistulosa*) at two cultivation depths. Both species produced measurable biomass under farm conditions, but responses were species- and depth-specific.

Water samples were not collected regularly at the Ouzinke array location so the decision was made to not process the samples collected at the Woody Island site. Also, Hobo data loggers were not deployed by farmers, as requested by project leads. While abiotic measurements are informative, their specific contributions to the observed phenotypic and yield responses can only be inferred. This experiment therefore focused on evaluating how cultivation depth influenced these outcomes.

For three-rib kelp, higher mean biomass at 2.2 m relative to 4.0 m supports the expectation that shallower depths provide more favorable conditions for growth, likely due to greater light availability. Morphometric traits, including blade width and midrib thickness, were relatively consistent across depths, suggesting that this species develops robust thalli under a range of conditions. From an industry perspective, three-rib kelp could be a candidate for commercial farming, particularly at shallow depths where higher yields are more reliable. Its relatively broad blades and sturdy midribs may also lend themselves to product diversification opportunities beyond those currently realized with sugar kelp (*S. latissima*).

In contrast, dragon kelp produced lower average yields overall, with modestly greater biomass at 2.2 m but higher frond density at 4.0 m. The inverse relationship between density and individual size suggests that depth influences establishment and morphology in ways that could complicate farm management. For commercial adoption, this implies that dragon kelp may require refined cultivation practices, such as controlling seeding density or pairing depth placement with local hydrodynamic conditions, to achieve predictable yields. Without such refinements, the risk of inconsistent production could limit its near-term viability as a farmed crop.

Cultivation of split kelp was unsuccessful, and the inability to confirm whether observed individuals originated from hatchery seed or wild set precludes interpretation of performance. This, is in fact, appears to be a common issue with split kelp as (1) it is notoriously hard to identify because it looks similar to several other single-bladed kelp species (e.g. can similar to *S. latissima*, *Laminaria digitata*, and *Laminaria longipes*) and may sometimes require DNA analysis for species confirmation (Franke et. al., 2023; Mauger et. al., 2021), and (2) producing seed for this species in the nursery is also proving challenging for operators, so far. For example, efforts to release spores for this species by the Native Conservancy, Prince William Sound Science Center, and University of Alaska Fairbanks (Juneau) in the autumn of 2025 have suggested that split kelp has a slow/trickling spore release behavior and also high carbohydrate exudate (i.e. slime) from the sorus tissues, perhaps affecting the success of spore settlement onto seed twine spools for nursery cultivation (*personal observation and communication by T. Stephens*). This spore release behavior could easily result in uneven or low settlement of spores due to spore abundance and entrapment in slime. Although reliable propagation was not demonstrated through this project, it stands to reason that this species would lend itself to farm cultivation as it is a common wild-set species on non seed portions of farms in the region (farmer communication).

Overall, these findings suggest that diversification of Alaska's kelp aquaculture industry will require careful evaluation of both biological performance and operational feasibility. *Three-rib kelp* shows promise as a viable addition to farm portfolios, particularly under shallow cultivation, and could help broaden product offerings. Dragon kelp, while ecologically abundant, may not yet be suitable for wide-scale industry adoption without methodological refinement. Split kelp likely requires further refinement of sori handling and spore release in a nursery setting before better understanding the performance in a farmed scenario. For a commercial sector that is currently reliant on a small number of species, these results highlight the importance of ongoing trials to identify new species that can both perform reliably under farm conditions and provide differentiated market opportunities.

Next Steps

It is difficult to determine how abiotic factors may have influenced the observed results, as this project was conducted during a single growing season with one outplanting period (Bischof et. al., 2019; Xiao et. al. 2019). Repeating the cultivation experiment across multiple seasons and outplanting times would strengthen the findings by revealing whether patterns of yield and phenotype at varying depths persist despite interannual variation in abiotic conditions such as light availability, nutrient concentration, salinity, and temperature (Stephens et. al. 2024). The 2024–2025 growing season was unusually dark, warm, and wet compared to previous years in the region (NOAA Climate Data).

Limited information is available regarding the biochemical composition of these kelp species (Hansen et. al., 2021; Holdt & Kraan 2011; Usov et. al., 2005;). Samples from this project were submitted for analytical testing through another SEC-funded initiative, *Joint Innovation Projects Cohort #2: Novel Compound Discovery in Newly Domesticated Alaskan Kelp.* Findings from that effort may identify potential industrial applications for the three species studied here or indicate which species have limited commercial relevance. These results will help refine the focus of future research on cultivation methods and their effects on yield and phenotype, as well as inform species selection for continued study.

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