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Neglected Natives: Distribution, Reproductive Biology and Early Life History of Two Endemic Sargassaceae

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Neglected Natives: Distribution, Reproductive Biology and Early Life History of Two Endemic Sargassaceae

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science in Molecular, Cell and Developmental Biology

by

Katelyn A. Alley

2026

ABSTRACT OF THE THESIS

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Master of Science in Molecular, Cell and Developmental Biology

University of California, Los Angeles, 2026

Professor Siobhan Braybrook, Chair

Southern California is home to native macroalgae in the Sargassaceae family (order Fucales); several function as canopy-forming species in lower intertidal and subtidal ecosystems and act as foundational species by providing food and habitat for an array of organisms. The two genera found in this family are *Stephanocystis* and *Sargassum*. Among these, two endemics are the focus of my studies, *Stephanocystis dioica* and *Sargassum agardhianum*, which are warm-temperate species whose natural range begins south of Point Conception. As ocean temperatures continue to warm in Southern California, such warm-temperate canopy-forming species may play an increasingly important ecological role as community structure shifts. Despite this, foundational knowledge, including their reproductive biology, remains poorly understood.

For *S. dioica*, I documented the reproductive phenology, identified the morphology of both sexes, and performed a series of experiments to culture embryos and juveniles. A novel *ex-situ* culture protocol successfully supported embryo and juvenile survival for up to 12 weeks. The reproductive phenology of *S. agardhianum* was documented establishing a summer-fall reproductive window. A morphological and histological study confirmed a monoecious habit and provided clear visualization of reproductive structures. These data provide a foundational tool for future research into the early life history and restoration potential of these native species.

For *S. agardhianum*, herbarium records, iNaturalist observations, and field surveys were used to investigate its past and present distribution in Los Angeles and Orange Counties. Populations in Orange County appear stable, potentially reflecting lower urbanization, reduced ocean discharge volumes, and lower human foot traffic. In contrast, in the Palos Verdes Peninsula (PVP; LA County) my surveys detected *S. agardhianum* at fewer locations than historically recorded, hypothesized to reflect anthropogenic stressors including decades of sewage discharge and human foot traffic. The northern edge of the range was found to be one site in PVP, shifted from historical Malibu. In contrast, populations in Orange County appear stable, potentially reflecting lower urbanization, reduced ocean discharge volumes, and lower human foot traffic. , making this site a key location for future work as our ocean warms.

Together, these studies provide foundational tools and baseline data for future research into the reproductive ecology, distribution, and conservation of these understudied warm-temperate native Sargassaceae.

The thesis of Katelyn A. Alley is approved.

Peggy Fong

Rachel Shahan

Siobhan Braybrook, Committee Chair

University of California, Los Angeles

2026

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LIST OF ABBREVIATIONS

PVP	Palos Verdes Peninsula
SCB	Southern California Bight
ASW	Artificial Seawater
SW	Seawater

ACKNOWLEDGEMENTS

This thesis represents the culmination of an eight-year journey back to academia, and it would not exist without the support, encouragement, and love of an extraordinary group of people. First and foremost, I am deeply grateful to my advisor Dr. Siobhan Braybrook, for taking a chance on me. As the first student in a newly reinstated master's program, I was very much a guinea pig, which she navigated with patience and unwavering support. She encouraged me to apply, trusted me with a project I am truly passionate about, and pushed me to become a better scientist through her brilliant scientific insight and feedback. She has been a great field buddy, always pulling me back to the task when I would get distracted by a cute marine animal! I could not have written a thesis I am proud of without her wisdom and dedication. I am endlessly grateful.

I want to thank my committee members, Dr. Peggy Fong and Dr. Rachel Shahan, for invaluable feedback. Dr. Shahan's kind and encouraging presence made this process more manageable, while Dr. Fong's direct and rigorous feedback challenged me to evolve as a scientific thinker. Her ecological expertise shaped how I interpret my research, and her willingness to write a reference letter that helped me land my dream job in Hawai‘i means more than I can express.

My lab mates Hugh and Jess kept me grounded through the hardest moments, joining me in the field and believing in me when I struggled to believe in myself. Hugh has become one of my closest friends, and I know our bond extends far beyond this degree. I am grateful to our lab manager Lauren, whose patience and brilliance made learning molecular techniques feel approachable rather than intimidating.

Isabel came into my life serendipitously as a volunteer and quickly became indispensable. She braved the 'danger zone' with me for the sake of seaweed and approached every responsibility with meticulous care and reliability. She is now a co-author for Chapter 1, having helped perform culture experiments and providing intellectual contributions. She is now a lifelong friend, and I could not have done this without her.

Dr. Steven Murray's foundational research inspired so much of my own. His generosity in opening his home, sharing decades of knowledge, and lending us an irreplaceable box of documents was extraordinary. His support at PSA and unwavering availability meant more to me than he knows; he truly has a heart of gold. I am grateful to my DSO Dr. Mike Anghera for his commitment to diver safety and his unmatched care for his divers. I want to acknowledge Chris Funada, whose freediving instruction, gear donations, and safety support during my fieldwork were invaluable.

My mom has been my greatest cheerleader throughout this entire journey. Her pride and encouragement carried me through my hardest days. I am grateful to my grandmother for her generous support of my physical wellbeing, and to my dad, stepmom, and brother for their patience and understanding. I also want to thank Dr. Jay Phelan for being an incredible professor and mentor. Teaching for his course deepened my passion for science communication and made me a better scientific thinker in ways that extend far beyond the classroom.

Finally, coming back to academia was itself a long journey. While working for Animal Mama, a domestic and wildlife rescue in Cambodia, I was exposed to the devastating realities of habitat loss and climate change, and I knew I needed to do

more. My supervisor Yulia Khuori told me she would put me on a plane if I decided to return to the US for a degree. I didn't let her, but her encouragement, her own inspiring academic journey, and her years of humanitarian work gave me the courage to take that leap. I would not be here without her.

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CHAPTER 1

An *ex-situ* embryo cultivation protocol for *Stephanocystis dioica*

This chapter will be submitted for publication as presented.

Preface

The following attributes work within this paper to the respective author(s)

Writing	K.A.A, S.A.B
Figures	K.A.A, S.A.B
Culture Experiments	K.A.A, I.C.C
Intellectual contributions	K.A.A, S.A.B., I.C.C

Chapter 1

An ex-situ embryo cultivation protocol for *Stephanocystis dioica*

Katelyn A. Alley, Isabel C. Carden, and Siobhan A. Braybrook

ABSTRACT

Southern California is home to native macroalgae in the Sargassaceae family, with many that function as canopy-forming species in lower intertidal and subtidal ecosystems. Among these, *Stephanocystis dioica* (*S.* dioica) is a warm-temperate species whose natural range begins south of Point Conception, where summer sea surface temperatures support vegetative growth and reproduction. As ocean temperatures continue to warm in Southern California, warm-temperate canopy-forming species such as *S. dioica* may play an increasingly important ecological role as community structure shifts. Despite this, methods for cultivation of *S. dioica* embryos and juveniles have not been described. Attempts to adapt culture protocols from related brown macroalgae to *S. dioica* were impeded by persistent contamination and low embryo survival, requiring systematic troubleshooting to identify an effective method. A simple sterilization and culture method was ultimately developed that successfully supported embryo development and juvenile survival. Here we present the first protocol for the generation and culture of embryos and juveniles for up to 12 weeks, providing a foundational tool for future research into the reproductive ecology and restoration potential of this native Sargassaceae.

Key index words: embryo; Sargassaceae; aquaculture; receptacle; conceptacle; dioecious; embryogenesis

INTRODUCTION

The Fucales order is home to 571 species worldwide (Guiry & Guiry 2026), including species from the genera *Fucus* and *Cystoseira* that have been well studied with respect to the order's diplontic life cycle (Yamanouchi 1909, McLachlan et al. 1971, Kropf 1997, Pearson 2006, Falace et al. 2018, Savonitto et al. 2019, Heesch et al. 2021, Rindi et al. 2023). Within Fucales, the majority of species are in the family Sargassaceae, including the widely distributed genus *Sargassum* and the recently classified genus *Stephanocystis,* the latter of which is endemic to the Pacific (Draisma et al. 2010).

Southern California is home to four *Stephanocystis* species: *S. dioica, S. osmundacea*, *S. neglecta*, and *S. setchellii* (Abbott and Hollenberg 1976). *S. dioica*, formerly *Halidrys dioica (Draisma et al. 2010)*, is endemic to the warm-temperate waters of the Southern California Bight, south of Point Conception. It is found on the Channel Islands and along the mainland coast (Abbott and Hollenberg 1976). This canopy-forming macroalga grows vegetatively in the late spring and early summer, is reproductive when oceans warm in the summer, and sheds its branches during the winter months leaving behind a perennial main axis (Setchell and Gardner 1925, Abbott and Hollenberg 1976, Lu and Williams 1994). As with other Californian Sargassaceae, early life history research on *S. dioica* has not been revisited since a cytology study in 1928 (Doubt 1928), with subsequent literature limited to a genetic diversity and population structure study (Lu and Williams 1994). Phylogenetic investigations later identified inconsistencies in the morphological systematics of North American Sargassaceae (Harvey and Goff 2006), ultimately leading to the reinstatement of the

genus *Stephanocystis,* into which *S. dioica* was transferred from *Halidrys* (Draisma et al. 2010).

As ocean temperatures in Southern California continue to rise, warm-temperate canopy-forming species such as *S. dioica* may play an increasingly important role in structuring rocky intertidal and subtidal communities as cooler-water species face increasing thermal stress (Bulleri et al. 2002, Thorne et al. 2024). Understanding the fundamentals behind *S. dioica*'s reproduction and early life history will improve our understanding of warm-water California natives and provide a foundation for future conservation or restoration efforts.

Here we present a protocol for the generation and culture of *S. dioica* embryos and juveniles up to 12 weeks, including information on collecting reproductive material in the field. This protocol is designed to be accessible and reproducible for researchers working with *S. dioica* or related Sargassaceae. Culture protocols from related Fucales species were used as guides and are recommended reading for researchers looking to adapt this protocol to other species (Siméon and Hervé 2017, Falace et al. 2018, Savonitto et al. 2019, Rindi et al. 2023). Details on equipment, reagents, and solutions for this protocol are provided in the Appendix.

METHODS AND RESULTS

Collecting reproductive algae

Observations and collections of *S. dioica* began in June 2025 (CDFW permit *S-250080006-25055-001*) in the Palos Verdes Peninsula in Los Angeles County where *S. dioica* can be easily found (See Figure A1.1 for map). Collecting was easiest during low tides in the lower intertidal zone, the upper limit of this species. At low tides it was easy

to identify sexes and access *S. dioica* to ensure no more than what is needed will be collected. During high tides, samples were also collected by diving; collecting methods should be determined based on collector experience and site conditions, which should be assessed for safety prior to and during collection.

At our sites, vegetative growth was observed beginning in spring, consisting of primary and secondary branches (Figure 1.1C); individuals ranged from what appeared to be new recruits or existing perennial main axes, consistent with known perennial life history of this species (Setchell and Gardner 1925, Abbott and Hollenberg 1976). Reproductive growth was observed from June-November 2025; however, timing may vary year-to-year with oceanographic conditions. The reproductive transition is preceded by an increase in branching order (tertiary) and by the development of pneumatocysts on secondary and tertiary branches (Figure 1.1 C). While vegetative branches were bright olive to yellow-green in color (Figure 1.1 A), the reproductive structures (receptacles) were consistently a darker reddish-brown coloration and did not exhibit the olive-green coloration of the vegetative branches (Figure 1.1 B), providing a useful field indicator of reproductive status.

Although *S. dioica* has historically been described as having “sexes indistinguishable by external morphological characters” (Setchell and Gardner 1925), receptacles of this dioecious diplontic species developed at the apical region of tertiary and quaternary branches and showed clear sexual dimorphism between male and female individuals (Figure 1.2). Male receptacles were thin and elongated with acute tips, while females displayed shorter, stockier receptacles (Figure 1.2 AB). Conceptacles appeared as distinct raised bumps along the receptacles (Figure 1.2 BC)

and housed the reproductive structures: either antheridia (male) or oogonia (female). Male receptacles were less pronounced compared to the rounded and conspicuous female conceptacle bumps (Figure 1.2 C).

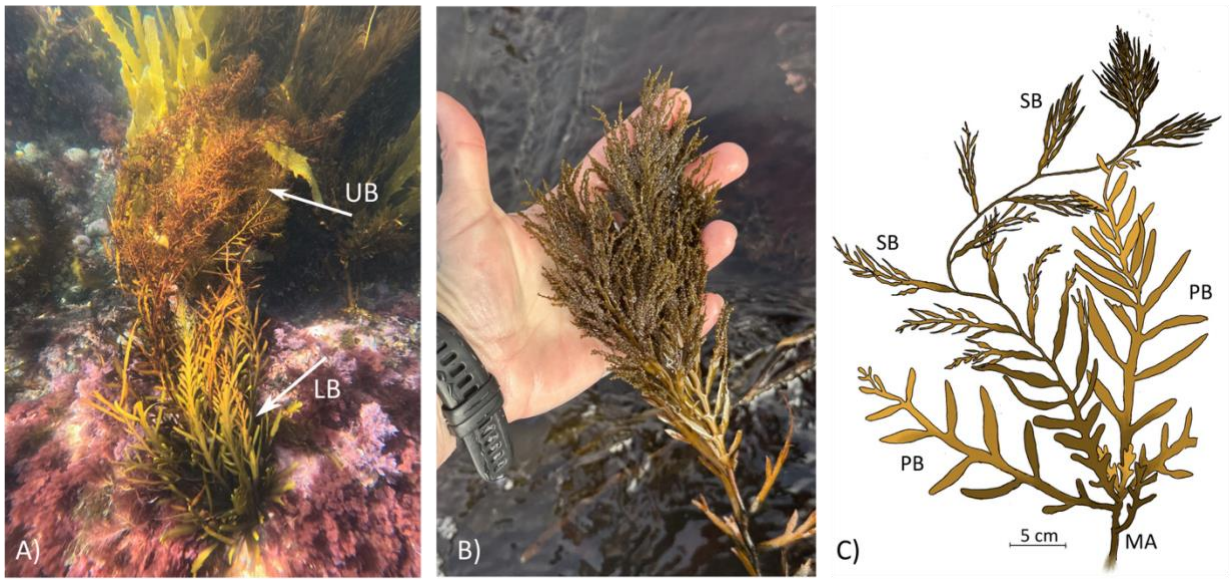

Figure 1.1

Vegetative and reproductive morphology of *Stephanocystis dioica* for field identification. **A)** Underwater photograph of a mature *S. dioica* thallus showing distinct lower (LB) and upper (UB) branch morphology, with bright olive to yellow-green vegetative coloration in the lower branches and darker reddish-brown receptacles visible at apical ends of the upper branches. **B)** Reproductive branch held in hand showing darker reddish-brown receptacles at the apical ends of tertiary branches, providing a useful contrast to the olive-green vegetative coloring. **C)** Drawing by Siobhan Braybrook. Labels indicate main axis (MA), primary branches (PB), and secondary branches (SB).

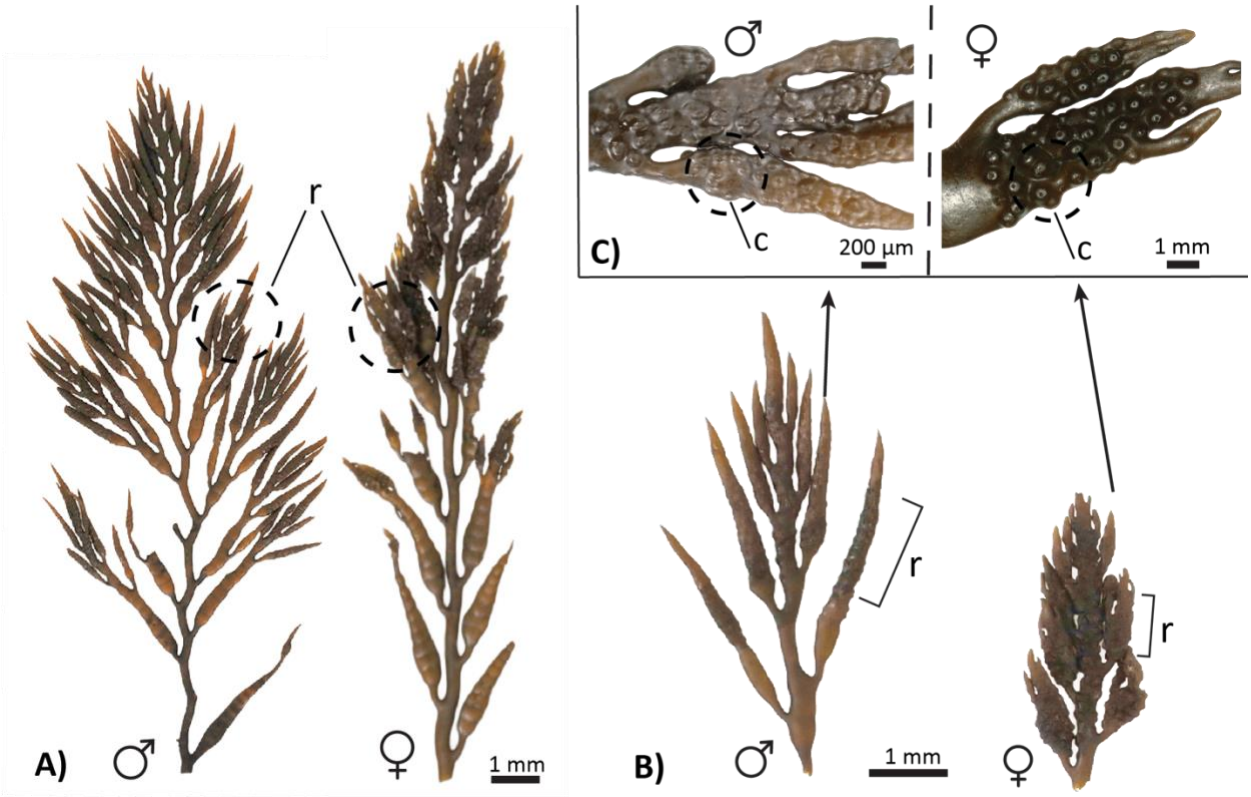

Figure 1.2

Sexual dimorphism in *Stephanocystis dioica* receptacle morphology. A) Male (left) and female (right) secondary branches bearing receptacles (r) denoted in dashed circles. **B)** Male (left) and female (right) tertiary branches with receptacles (r) extending onto quaternary branches. **C)** Close up of male (left) and female (right) receptacle surfaces; female conceptacle (c) bumps are more rounded and conspicuous than male, highlighted with dashed circles.

A single secondary branch loaded with receptacles of each sex was enough for multiple technical replicate fertilizations (Figure 1.2 A). We removed secondary branches using a cutting tool, leaving the individual intact in the field. The thallus was checked for visible epibionts, which were gently removed by shaking the branches in seawater or plucking by hand. Epibiotic polychaetes were occasionally found firmly adhered to the branch rachis (midrib) beneath a mucus layer and were avoided during collection. Branches were stored in separate plastic, labeled, resealable bags to ensure no mixing of individuals or sexes and avoid early fertilization. We added a small amount

of seawater to keep the samples hydrated but not submerged. Samples were stored in a cooler for transport and transferred into a refrigerator within 1-3 hours after collection. We usually prepared the receptacles the same day as collection; however, on occasions when samples were stored overnight in a refrigerator, gamete release was also successfully achieved.

Preparing receptacles

Once back in the lab, a quick sex-verification can be done to ensure that field sex determination yielded enough male and female receptacles. Using a razor blade, hand sections were made as thin as possible; optimal section thickness is skill-dependent and may require practice, but sections should be thin enough to allow light transmission through the conceptacles for gamete visualization. Hand sections were transferred to a microscope slide using fine forceps, then a drop of Artificial Seawater (ASW; e.g. Instant Ocean), was added with a cover slip placed over the section. Conceptacles were examined under a dissecting microscope with a 10x-16x objective. Male conceptacles showed clusters of light-colored antheridia (Figure 1.3 AB). Female conceptacles had large, dark oogonia and eggs (obvious spheres; Figure 1.3 CD).

Once sex was confirmed, receptacles to be used for fertilization were selected. Using a stiff brush, receptacles were scrubbed under fresh water to remove additional epibionts. The branches were rinsed in ASW and placed on a paper towel until all the samples were scrubbed & washed. Multiple sterilization options were evaluated (data not shown), including iodine, bleach, and ethanol at various concentrations; however, the method presented here resulted in consistent gamete release and highest subsequent embryo survival.

Note: When adapting for species with little microscopic documentation, we recommend fixing, embedding, and sectioning receptacles to confirm hand-section results at the beginning of protocol development. See Figure A1.2 for example images.

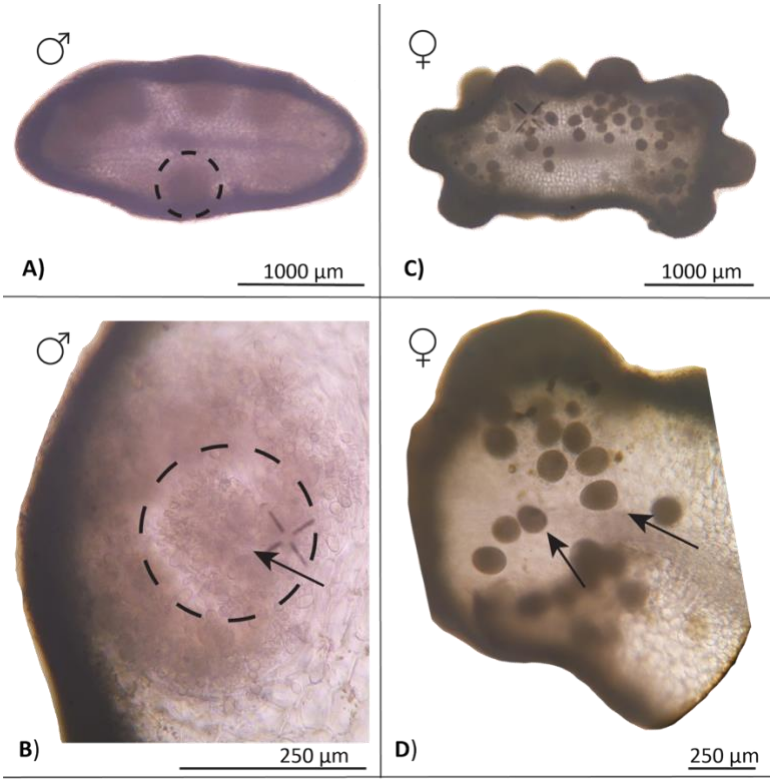

Figure 1.3

Hand sections of male and female *Stephanocystis dioica* receptacles viewed under a dissecting microscope. A) Male receptacle with a conceptacle (dashed circle). **B)** Male conceptacle showing light-colored antheridia cluster (dashed-circle, arrow). **C)** Female receptacle showing large, dark oogonia. **D)** Female conceptacle with dark, spherical oogonia at various developmental stages (arrows).

Once all branches were scrubbed and washed, each branch was rolled into 1-2 fresh paper towels and firmly pressed to remove excess water. Each branch was then rolled individually into a fresh paper towel, ensuring branches remain lightly damp, and stored in labeled resealable bags inflated with ambient air. Because *S. dioica* is dioecious, one male and one female individual were combined into a single bag, constituting one biological replicate, defined here as a unique male-female pairing

representing one genetically distinct cross. Multiple bags therefore represented multiple independent biological replicates, each consisting of a different male-female pairing. Bags were stored at a cool temperature (14C-20^{o}C; a refrigerator or a cool incubator was sufficient) for a minimum of 3 days and a maximum of 7 days.

Note: Skipping this desiccation step resulted in poor gamete release and low survival of subsequent embryos

Fertilization

The workbench was prepared by spraying with 70% ethanol and wiping the surface. One biological replicate was removed from the incubator and unwrapped. A razor blade was used to remove tertiary branches from the secondary branch (Figure 1.2 B). One male and one female tertiary branch were placed in a labeled 250 mL beaker and covered with plastic wrap until all samples were prepared, with one biological replicate per beaker. An additional cut was sometimes necessary in the middle of the tertiary branches to promote circulation within the beaker; however, excessive cuts were avoided as this increased mucilage and debris, complicating subsequent filtering steps.

An aquarium pump with a tube and filter leading to each beaker (Figure A1.3) was assembled and 100 mL of Enriched Natural Seawater was added (ENSW; see Appendix 1, Reagents and Recipes). The pump was set to maximum speed (Sun YT-304, Sunsun) to ensure sufficient mixing and agitation at room temperature, under natural ambient light. Fertilization usually occurred within 1 hour, but continued for up to 3 hours or overnight as more gametes were released. Fertilization progress was checked by extracting a small amount of the beaker fluid, hereafter referred to as the

fertilization mixture, with a pipette and placing it on a microscope slide for observation. Approximately 24-48 hours after fertilization, embryos formed apical and basal poles and showed a teardrop shape (Figure 1.4).

Note: Fertilization was asynchronous under this protocol; achieving synchronization would require more optimization beyond the scope of this paper.

Figure 1.4

***Stephanocystis dioica* embryo development following fertilization.** Images shown left to right at approximately 24, 48, 72, and 96 hours and one-week post-fertilization initiation, viewed under a dissecting microscope. At 24 hours, early apical-basal polarity is visible; at 48 hours, a distinct teardrop morphology is apparent; at 72 hours, early elongation is visible; at 96 hours, early rhizoid extension is apparent; at one week, rhizoids are fully developed with visible cell divisions in the embryo. *Note: fertilization was asynchronous, so timepoints are approximate.*

Each fertilization mixture was strained through a ~ 2mm^2 metal strainer over a clean 100 mL beaker to remove receptacles and other debris. Plastic filters were not used as zygotes attached to the filter surface, diminishing yield. After straining, the density of zygotes was checked by placing a small amount of the fertilization mixture on a glass slide and observed with a dissecting microscope.

Note: In our experience natural seawater appeared to support faster and more robust embryo growth than artificial seawater; however, this was not formally quantified but may be helpful for readers when optimizing their workflow.

Embryo and juvenile culture

Once fertilization was confirmed, 1 mL of strained fertilization mixture was pipetted into a sterile 60mm x 15mm round petri plate, repeating as desired for technical replicates, defined here as multiple culture dishes established from the same biological replicate. 7 mL of ENSW was added to each dish and incubated under conditions reflecting the natural environment of collection (for our collections: 20C, 650-800 lux, 14L:10D). Water changes were performed every seven days using ENSW. Within one week, embryos produced rhizoids and adhered to the bottom of the plate (Figure 1.4).

Culturing in 60mm x 15mm Petri plates sustained juveniles for 6-8 weeks (Figure 1.5). For longer culture periods, Petri plates containing juveniles were transferred to larger vessels (e.g. 190ml plant tissue culture containers). Aeration was provided using the same aquarium pump and tumble setup described for fertilization. Using this method, juveniles were maintained for at least 12 weeks, the duration of our experimental timeline.

Note: Epiphytic Ectocarpus spp. were commonly observed in culture dishes and would engulf juveniles when cultures were maintained beyond 12 weeks.

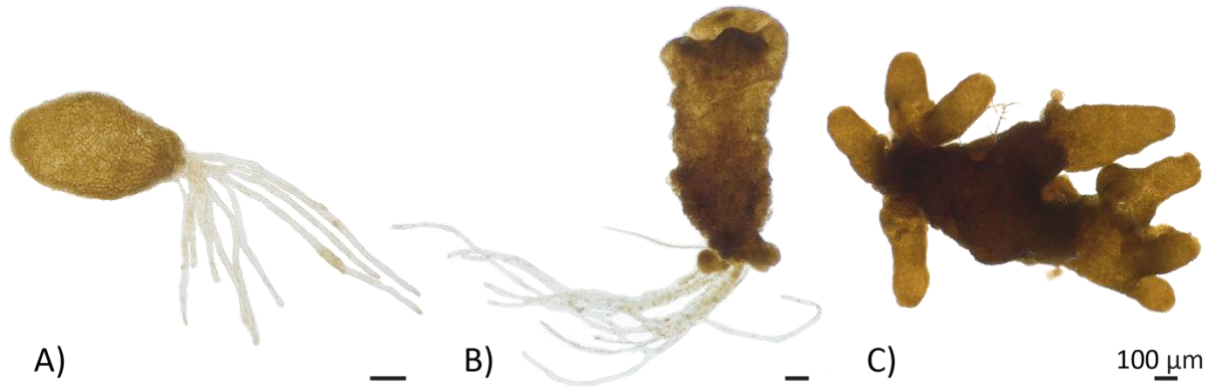

Figure 1.5

***Stephanocystis dioica* juvenile development in culture A)** Two-week old juvenile with extensive rhizoid development. **B)** Five-week-old juvenile showing increased embryo size with continued **rhizoid**

development. **C)** Six-week-old juvenile displaying complex branching; rhizoids were not visible beneath the embryo tissue. Scale bars = **100 µm**.

CONCLUSIONS AND UTILITY

Here we present a protocol for the generation and cultivation of *S. dioica* embryos and juveniles, with success up to 12 weeks. This protocol can be used to further investigate embryo development at early life stages by synchronizing gamete release to track growth and development. Future experiments can place receptacles of each sex into separate beakers for gamete release, followed by combining segregated gamete media into a new beaker. This protocol can be used to explore potential photo polarization cues for *S. dioica.* Future trials can experiment with substrate types, with rock and rough clay tiles suggested for out planting trials. In addition, this protocol can be used to explore other potentially economic uses of this species, since other Sargassaceae have demonstrated commercial value (Peng et al. 2024).

APPENDIX 1

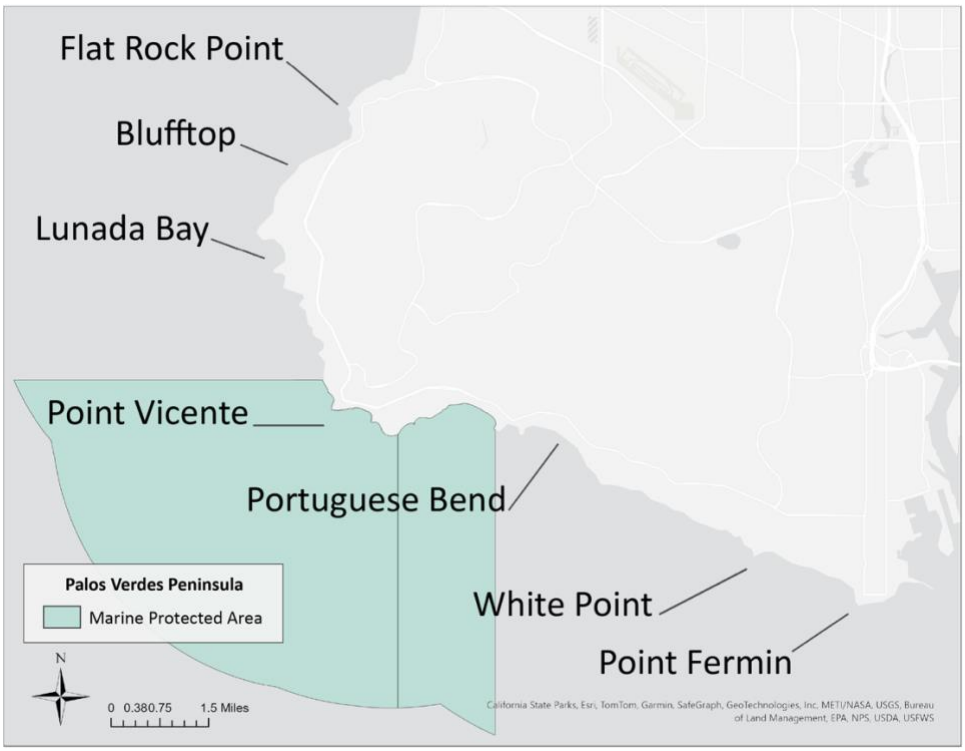

Figure A1.1

Map of *Stephanocystis dioica* collection sites on the Palos Verdes Peninsula, from Torrance to San Pedro. *S. dioica* was collected at Blufftop, Lunada Bay, and Point Fermin (CDFW permit *S-250080006-25055-001*) for embryo culture experiments described in this protocol.

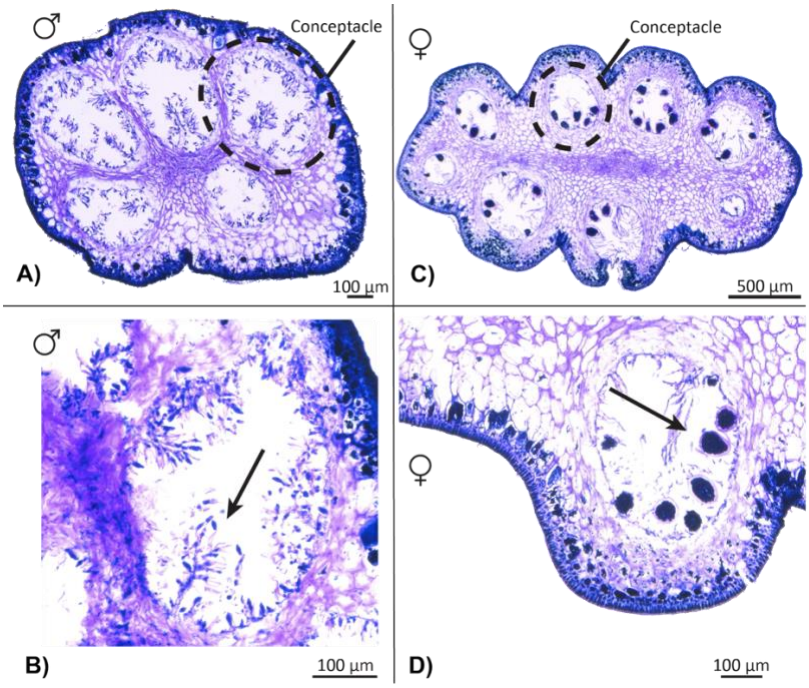

Figure A1.2

Cross sections of *Stephanocystis dioica* male and female reproductive structures. Samples fixed in 4% formaldehyde, embedded in paraffin wax, and sectioned at 10 µm; sections stained with Toluidine Blue O (TBO) and imaged using a Keyence VHX-7000 microscope. **A)** Male receptacle with conceptacle (dashed line). **B)** Male conceptacle with antheridia (arrow). **C)** Female receptacle with conceptacle (dashed line). **D)** Female conceptacle with oogonia (arrow).

Figure A1.3

Aquarium pump and filter setup used for gamete mixing during fertilization. Sunsun YT-304 aquarium air pump set to maximum power (8.5W), with one tube fitted with a 0.22 µm syringe filter per fertilization beaker.

Table A1.1

Laboratory supplies and equipment used for *Stephanocystis dioica* embryo and juvenile culture.

Laboratory Supplies
Single edged razor blade
60mmx15mm round petri plates (Fisher Scientific, catalog number: FB0875713A)
Aquarium airline tubing (NACX, catalog number: B07THGJCXS)
3. Air filters LAUREN (tumble culture supplies)
250 mL plastic beakers
100 mL plastic beakers
200 um metal strainer
Re-sealable plastic bags
Paper towels
Basic microscope slides

1 mL disposable Pasteur pipettes
Fine forceps (Cat# and company, LAUREN Dupont)
Basic 15x15mm cover slips
Nalgene Rapid-Flow Sterile Disposable Top Filters (Thermo-Scientific, catalog number: 09-740-22K)
190 mL plastic plant tissue culture containers (Greiner Bio-one, catalog number: 967161) Check w Lauren
P1000 pipette and tips
P200 pipette and tips
P200 pipette and tips
Note: The number of beakers will be determined based on the number of biological replicates.
Equipment
Dissecting Microscope (e.g. Zeiss ZT20, Zeiss)
Incubator capable of providing 20C and 650-800 Lux white light
Aquarium pump (**SunSun**) LAUREN
Mesh collecting bags
Intertidal footwear
Cooler
Utility knife or dive knife for cutting branches in the field
Autoclave

REAGENTS AND RECIPES

Reagents

1. F/2 Algae Food Part A & Part B (Fritz Pro Aquatics) (Filter sterilized)

2. Penicillin G (Sigma-Aldrich)

3. Streptomycin (Sigma-Aldrich)

4. Chloramphenicol (Sigma-Aldrich)

5. Antibiotic Antimycotic Solution (100x) (Sigma-Aldrich, catalog number: A5955-100ML)

6. Germanium dioxide, 99.999% (Fisher Scientific, catalog number: AA1115506)

7. Instant Ocean (Instant Ocean, catalog number:169526)

8. Ultrapure water (MilliQ, 18.2 MΩ·cm, Millipore/MilliporeSigma)

Recipes

1. Triple Antibiotic solution (3AB)

Triple antibiotic stock solution: Add 1 g of penicillin G and 0.5 g of streptomycin to 9mL deionized water and mix to dissolve. Add 0.1 g of chloramphenicol to 1mL of ethanol and mix to dissolve. Add the 1 mL chloramphenicol to the 9 mL of strep and Penicillin G for a total stock volume of 10 mL, mix and filter through a 0.22 µm membrane, aliquot and store at −20°C. Add 0.5 ml/L seawater (SW) to achieve a 1:2000 working dilution (Le Bail and Charrier 2013).

Reagent	Stock concentration	Working concentration
Penicillin G	100 mg/mL	50 µg/L
Streptomycin	50 mg/mL	25µg/L
Chloramphenicol	20 mg/mL	5µg/L

2. Saturated GeO2 solution

Saturated GeO2 is made by dissolving 0.894 g in 200 mL MilliQ water until saturation: place on stir place and stir to dissolve at least overnight but up to 24 hours. After maximum dissolving (some particles may be left undissolved) filter sterilize (0.22 µm

membrane) the solution into a sterile bottle. Add 500µl/L for SW for 1:2000 working dilution (Shea and Chopin 2007).

Reagent	Stock concentration	Working concentration
Germanium dioxide	Saturated (~4.5 mg/ml)	~2.25 µg/L

2. Enriched Natural Seawater (ENSW)

Natural seawater should be filtered with a 0.2 µm filter to remove any impurities and autoclaved for sterilization. To 1L of sterilized NSW: Add 150 µL of F/2 A and B, 500 µL of saturated GeO2, 500 µL of 3AB, 1 mL of AAM (1:1000 dilution; 0.1X working) and invert bottle to mix solution. ENSW can be stored in a fridge or incubator (14C-20C) for long term use.

Reagent	Stock concentration	Volume per liter
F/2 A		132.5 µL
F/2 B		132.5 µL
3AB	0.5 mL/L	500 µL
AAM	100X	1 mL
GeO2	500 µL/L	500 µL

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CHAPTER 2

Historical and Contemporary Distribution, Reproductive Phenology, and Morphology of the Southern California Endemic *Sargassum agardhianum*

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INTRODUCTION

California is home to the highest number of plant and animal species in the US and is recognized as a global biodiversity hotspot (Dobson et al. 1997, Myers et al. 2000). This diversity extends to its marine flora, with at least 669 species of red, green, and brown seaweeds reported along its coast (Abbott and Hollenberg 1976), including roughly 150 species of brown macroalgae (Miller 2024a). Southern California, in particular, supports high seaweed diversity due to the mixing of cold waters from the California Current and warmer waters from the Southern California Countercurrent or Eddy, along with nutrient enrichment brought by wind-driven coastal upwelling (Littler 1980, Littler et al. 1991). However, these macroalgae can be susceptible to anthropogenic effects leading to changes in diversity over time, potentially dependent on site-specific factors/impacts (Littler and Murray 1975, Murray et al. 1999, Murray and Littler 1984).

In mainland Los Angeles County there has been a consistent increase in urbanization and the human impacts that brings, such as foot traffic and pollution (Commoner et al. 1971, Murray et al. 1999, Thompson et al. 2002). When combined with more global changes in ocean parameters such as temperature and salinity, there are a considerable amount of anthropogenic factors testing Southern California's intertidal macroalgae (Crowe et al. 2000, Thompson et al. 2002, Lucas and Smith

2016). In order to understand the impacts of anthropogenic effects on our macroalgae, we must first understand their historical and current distributions and temporal trends, followed by specific understanding of a species' reproductive phenology and development which interact with such effects. This fundamental information is necessary to further understand the dynamics between species distributions and human impacts and will be the focus of this chapter.

Southern California's Brown Macroalgae

The brown macroalgae of California includes species in the order Fucales (rock weeds) that contribute to coastal ecosystem function and health (Bolton 1996, Bertness et al. 1999, Bulleri et al. 2002, Sapper and Murray 2003). Macroalgae within the order Fucales tend to predominate rocky intertidal areas and many act as foundational species by providing food, habitat, and an environment for nursery and spawning for an array of organisms (Bertness et al. 1999, Bulleri et al. 2002, Roy et al. 2003, Sapper and Murray 2003, Whitaker et al. 2010, Fales and Smith 2022a).

The Sargassaceae family shows increasing species diversity as one moves south towards lower latitudes and this is particularly evident in the genus *Sargassum* (Abbott and Hollenberg 1976). The Southern California Bight (SCB; encompassing the area south of Point Conception through Baja California (Southern California Coastal Water Research Project 1973) is home to two endemic *Sargassum* species: *Sargassum agardhianum* Farlow and *Sargassum palmeri* Grunow, with *S. agardhianum* being the only mainland species (Abbott and Hollenberg 1976). There are also two introduced species, *Sargassum muticum* and *Sargassum horneri*, which been the topic of extensive study with respect to their introduction, distribution, life history, and phenology

(Deysher and Norton 1981, Ambrose and Nelson 1982, Wilson 2001, Monteiro et al. 2009, Baer and Stengel 2010a, Kerrison and Le 2016, Smith 2016); however, there are gaps in such foundational knowledge for SCB endemic *Sargassum* species.

Sargassum agardhianum and its relatives

This study is focused on *S. agardhianum*, the only SCB endemic *Sargassum* present on the mainland coast (Abbott and Hollenberg 1976) and therefore likely the most exposed to urbanization impacts. *S. agardhianum* was first described by J.G. Agardh (1889) and further characterized by Setchell and Gardner in 1925 as having 25-35cm long fronds, sharply toothed margins on the blades, with receptacles in clusters around the axils, simple or with sharp projections with conspicuous conceptacles (Agardh 1889, Setchell and Gardner 1925). The type locality for *S. agardhianum* is San Diego, California, with isotypes accessible online at the University of California Herbarium (UC231460, UC687929, and UC99516).

The reproductive structures, antheridia and oogonia, were described as often being “in separate conceptacles in the same individual or in the same receptacle” (Setchell and Gardner 1925, p.717), indicating that *S. agardhianum* is monoecious. When Hollenberg and Abbott published the Marine Algae of California (1976), *S. agardhianum* was described to be “reduced in the winter, often forming dense stands in the lower intertidal to upper subtidal.” Beyond these descriptions, there is little known about its reproductive phenology, its reproductive structures, or its current distribution.

There are six other *Sargassum* species in the SCB, endemic to Baja California (Andrade-Sorcia et al. 2014), that have recently been shown to be closely related to *S. agardhianum* (Yip et al. 2020); while also lightly represented in the literature, they do

share some traits with *S. agardhianum*. For example, *S. agardhianum* and the species of *Sargassum* in Baja have similar blade and receptacle descriptions, are all monoecious, and exhibit similar intertidal zonation (Andrade-Sorcia et al. 2014, McCourt 1984a, McCourt 1984b).

The reproductive phenology of the Baja *Sargassums* indicate that they are fertile once reaching maximum size in the spring, shedding their receptacles in the summer (McCourt 1984b). Given their relatedness and similarities, it is likely that *S. agardhianum* will display a similar reproductive window, although it may be shifted due to differences in environmental conditions in LA County compared to Baja; it should be noted that the Baja *Sargassum sinicola*, an exception, has two reproductive windows, one in spring and another in fall (McCourt 1984b). Thus, information on the Baja *Sargassums* may be useful in determining the reproductive phenology of *S. agardhianum*, and descriptions of its reproductive strategies.

Setchell and Gardner (1925) noted that *S. agardhianum* could be found in both types of habitat and that this influenced the algae's form; they described *S. agardhianum* to grow short and stocky when always covered with water (exposed areas) and more elongated and thinner in quieter waters. This difference in morphology was previously observed for related Baja *Sargassum*s*: Sargassum sinicola* and *S. herporhizum* (McCourt 1984b). Since the Baja Sargassums are well covered during high tides, McCourt (1984b) hypothesized that the positive correlation between branch length and pool depth seen in Baja Sargassums was related to their experienced depth at low tide.

<u>Southern California Survey History and Changes in Intertidal Macroalgae</u>

In 1956, E. Yale Dawson, for the California State Water Pollution Control Board, conducted an extensive oceanographic survey of Southern California that included benthic intertidal macroalgae (Dawson 1959, 1965). Dawson surveyed 44 sites solely along the SCB, with 42 on the mainland, one on Santa Catalina Island, and one on San Clemente Island. Dawson concluded that many species had decreased in abundance since the documentation generated in the late 1890s and early 1900s by W.A. Setchell & N.L. Gardner (Dawson 1959, 1965), attributing the loss to sewage waste and general anthropogenic activity. Over the next four decades, researchers revisited Dawson's sites to further examine changes in intertidal macroalgae over time.

Between 1973 and 1974 Thom and Widdowson surveyed 42 of Dawson's mainland sites and ultimately concluded that there had been little change in Los Angeles County sites since Dawson, with the exception of White Point (a slight increase in species) and Point Fermin (a slight decrease in species) in San Pedro. They attributed their results to an equilibrium achieved between the intertidal organisms and human disturbance from visitation and pollution, maintaining reduced, but stable, populations (Thom and Widdowson 1978).

Littler & Murray conducted an extensive three-year sampling program (1975-1978) of intertidal communities on the mainland and islands of the SCB, revisiting many of Dawson's survey sites (Littler and Murray 1974, 1975, Murray and Littler 1978, 1984, Littler and Littler 1984, Littler et al. 1991); however, since Littler & Murray employed a distribution and abundance procedure, differing from Dawson's technique, it is difficult to compare their data (Littler and Littler 1984). With respect to anthropogenic impacts, Littler and Murray (1975b) observed both species loss and stability at a San Clemente

site close to sewage discharge, indicating differential macroalgal susceptibility to such outflow (Littler and Murray 1975).

In a later study at White Point, near the ocean outfalls, Littler & Murray (1984) assessed the macrophyte community & described a dominance of turf forming crustose and jointed calcareous species and a shortage of thick leathery species. These early successional species were described to quickly rebound after disturbances, have low productivity, and have adaptations to deter herbivory (Littler and Murray 1974, Murray and Littler 1984). It was concluded that White Point had experienced a shift from larger macroalgal species towards turf and crustose species (Murray and Littler 1984).

The macrophyte communities of the Palos Verdes Peninsula (PVP), here described as the coastline extending from Palos Verdes through San Pedro, have received few investigations since the surveys of Dawson, Thom & Widdowson, and Littler & Murray (Harris 1983, Gerrard 2005). This region has experienced environmental changes driven by decades of sewage discharge and associated contaminants along the coast (Littler and Murray 1975, Grigg 1979, Murray and Littler 1984). Subsequent regulatory changes, including the Clean Water Act of 1972 and the ban on DDT and PCBs, may have contributed to shifts in intertidal macrophyte communities since these surveys were conducted (Grigg 1979, Taylor and Talavera 2018). In addition, ocean warming has been strongly correlated with shifts in Fucales species distributions around the world (Duarte et al. 2013, Smale and Wernberg 2013, Thomsen et al. 2019) and in California (Fales and Smith 2022b, Wood 2023).

S. agardhianum Observations and Anthropogenic Impacts

In the surveys above, Thom documented *S. agardhianum* at Portuguese Point in 1973 and Littler and Murray on San Clemente Island throughout their studies (Littler and Murray 1974, 1975, Thom and Widdowson 1978, Thom 1980, Gerrard 2005); however, it was not explicitly examined or noted in any of the other surveys.

In their work on San Clemente Island (1975), Littler & Murray found that S*. agardhianum* was growing no closer than 10m to a sewage outfall indicating that this species may be particularly sensitive to such outflow. Given the warming trends for the Pacific Ocean, the occurrence of several extreme heat events in Southern California since 2000 (Fumo et al. 2020), and the continued concern over pollutants in marine water and sediments in LA County, specifically the PVP that frequently faces landslide movement (Pondella et al. 2018, Palos Verdes Peninsula Watershed Management Group 2019), it is possible that *S. agardhianum* populations have shifted or are no longer detectable in some locations on mainland California over the last several decades.

In this study, our objectives were to (1) compile historical and recent observations of *S. agardhianum* in LA and Orange Counties, (2) document the current range of *S. agardhianum* within mainland Orange and LA Counties, with an emphasis on the Palos Verdes Peninsula, and (3) determine the reproductive phenology, describe the reproductive habit, and characterize morphological variation in branch length across several populations of *S. agardhianum*. We hypothesize that the recorded distribution of *S. agardhianum* in mainland LA County has changed since historical surveys.

METHODS

Curating & Mapping Observations from Herbaria Records & iNaturalist

Herbarium records were accessed through online databases from the University Herbarium at UC Berkeley (University Herbarium 2025) and iDigBio (iDigBio 2025) and in-person records were examined at the UCLA Mathias Botanical Garden Herbarium. Since many of the vouchers for *S. agardhianum* originated before the use of GPS, records were hand-curated to refine herbaria-provided GPS coordinates as follows: For specimen labels that included more detail in collection locality (i.e. beach or point name), coordinates were picked at random within that area; when labels were general (i.e. with only “San Pedro” or “Palos Verdes” as the location found), the coordinates provided by the herbaria databases were retained (n=56); more modern specimens retained their herbaria-provided coordinates as well, after verification. Duplicate specimens were removed for this analysis (n=21). Specimens or digital photographs were visually inspected to verify the species identification before being included in subsequent analysis. The iNaturalist database was queried for *S. agardhianum* observations in LA and Orange Counties (under “Place”) and data were downloaded for curation. Observations were hand-curated for species identification accuracy (often conflated with *S. muticum*) and removal of observations that likely originated from beach wrack (drift pieces of algae found on the shore). All curated observation data (herbaria & iNaturalist) were mapped in ArcGIS Pro software.

Present Survey

Scientific survey & collection permits were obtained from California Department Fish & Wildlife (CDFW Permits: S-250080006-25037-001, S-250080006-25055-001), with all surveys conducted between April 2025 and April 2026.

Surveys were first conducted at known populations in Orange County to characterize *S. agardhianum* habitat and co-indicator species, which informed PVP surveys and provided a comparison for non-marine protected area (MPA) sites in the Palos Verdes Peninsula. Initial surveys were conducted in Crystal Cove and Laguna Beach (in the Crystal Cove State Marine Conservation Area [SMCA] and the Laguna Beach State Marine Reserve (SMR) and Laguna Beach SMCA MPAs, respectively) during the winter and early spring months when the perennial *S. agardhianum* is described as being in a shortened vegetative state (Setchell and Gardner 1925, Abbott and Hollenberg 1976).

A thorough search was conducted at 9 of 10 planned sites in the PVP study region (See Table 2.1) to determine if *S. agardhianum* was present; one planned site, Portuguese Bend, was inaccessible due to landslides. Intertidal surveys were conducted by following the natural structure of the intertidal to cover from the high-to-middle intertidal to the lower intertidal/upper subtidal zones. A switchback pattern was followed perpendicular to the shoreline to be as thorough as possible. Field searches were planned around the predicted tide calendars (National Oceanic and Atmospheric Administration 2025), with low tide searches conducted with careful footing to minimize disturbance. Other canopy algae (such as *Egregia menziesii*) were lifted to ensure *S. agardhianum* growing beneath was not missed. During high tide days, surveys were carefully conducted via snorkeling and freediving. Site access was checked prior (City of Rancho Palos Verdes 2024) due to frequent closures from landslide activity. Species observations were logged in ArcGIS Field Maps.

Length of time and number of visits to the PVP sites were site-specific, influenced by factors such as existing available substrate and preferred intertidal zones, along with observed populations of other algae and intertidal species (See Appendix Table A2.1). If habitats were observed to be dominated by disturbance-tolerating species for several meters, they were only surveyed once, as the dominance of such species suggested recent disturbance has occurred. Since *S. agardhianum* is not early successional (Littler and Murray 1975), its presence in such areas was considered unlikely (Littler and Murray 1975, Murray and Littler 1978).

Assessing Branch & Reproductive Morphology of *S. agardhianum*

Branch length has been shown to vary with habitat type in related Baja *Sargassums*, potentially reflecting differences in hydrodynamic exposure and submersion time (McCourt 1984b). To assess the variability of branch length across populations, 1x1m quadrats were placed haphazardly within a contiguous patch of *S. agardhianum*. The number of quadrats per site was determined by available existing patches: five quadrats at Blufftop (n=125, PVP, LA County), five at Crystal Cove (n=125), three at Shaws Cove (n=75, Laguna Beach), and one at Goff Cove (n=25, Laguna Beach). Each quadrat was divided into five sections, and five primary branches were measured per section. Primary branch length was measured in centimeters from the holdfast to the branch tip.

Differences in median branch length between each site and Crystal Cove were assessed using a randomization test in the Plots of Differences Shiny application (Goedhart 2019), with Crystal Cove designated as the reference site. The randomization test works by repeatedly shuffling site labels across all observations to

simulate differences by chance alone, and the p-value represents the proportion of 1,000 randomizations that produced a difference equal to or greater than the observed value. Branch length distributions were visualized using ggplot2 (Wickham 2016, R Core Team 2024).

Detailed survey notes were voice recorded and written notes were later transcribed. These notes included biotic observations such as observed intertidal marine species and other algae species, especially those also belonging to the Sargassaceae family, both native and recently introduced species.

To determine the reproductive phenology, specimens were collected during the surveys to assess for reproductive branches (receptacles) that house the gametes within circular cavities known as conceptacles. Survey dates were determined by tidal conditions and logistical availability, with surveys concentrated during the hypothesized summer-fall reproductive window based on historical descriptions (Abbott and Hollenberg 1976, Miller 2024b). Individuals were carefully removed by hand or with a utility knife above the holdfast and placed into sealable plastic bags which were transferred into a cooler for transportation back to the laboratory. Three herbarium pressings for each site where *S. agardhianum* was collected were prepared and are deposited at The University Herbarium (UC Berkeley), the UCLA Mildred Mathias Herbarium, and the Braybrook Lab (UCLA).

Algae were rinsed with freshwater, lightly scrubbed, and epiphytes were removed with forceps to better visualize the axils. A dissecting microscope was used to detect the presence or absence of receptacles. Imaging of reproductive branches, receptacle axils,

and individual receptacles were obtained with a Keyence (VHX-7000, Keyence) microscope.

For histology, individual receptacles and/or complete axillary branches were fixed in 4% formaldehyde in ultrapure water, inside a vacuum dome overnight. Samples were washed with ultrapure water three times for 15 minutes and then dehydrated in an ethanol series before transitioning to Histoclear (HS-202) and finally into a Paraplast (19216) Paraffin wax and molds. 10 µm serial sections of the receptacles were obtained using a microtome (RM2165, Leica). Sections were dewaxed in Histoclear (HS-202) and rehydrated with an ethanol series and phosphate-buffered saline (PBS; 137mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.8mM KH_2PO_4, 1mM $CaCl_2$, 0.5mM $MgCl_2$) before staining with 0.05% Toluidine blue O in PBS. Imaging was conducted using a Keyence (VHX-7000, Keyence) microscope.

RESULTS

Reduction in *S. agardhianum* Observation Records Over Time

Given the sparse observation record of *S. agardhianum* in the SCB survey work introduced above, we turned to publicly available observation records from herbaria and iNaturalist to determine where and when *S. agardhianum* had been observed in Los Angeles and Orange Counties.

We identified four major time blocks of observations within our curation: Early era records (1896-1941), Dawson era records (1942-1959), Intermediate era records (1960-1986), and Modern era records (2007-2025), with the number of recorded observations varying across time (Figure 2.1). ‘Early era records’ document observations of *S. agardhianum* in Southern California beginning in the 1880s, with the earliest reports

covering Los Angeles and Orange counties, including Santa Catalina Island. In the later part of the Early era, researchers and surveyors for the Allan Hancock Foundation at the University of Southern California (USC) contributed to observations throughout the 1920s and 1930s, providing a strong observation record in both counties for the period (Figure 2.1 A; n=89).

Figure 2.1

Distribution of *Sargassum agardhianum* observations in Los Angeles and Orange Counties across four time periods. A) Early era (1885-1941, 56 years): observations depicted as circles with a red color gradient from dark (1885) to light (1941). **B) Dawson** (1942-1959, 17 years): observations depicted as circles with an orange color gradient from dark (1942) to light (1959); triangles indicate Dawson's survey observations. **C) Intermediate** (1960-1986, 26 years): observations depicted as circles with a green-to-blue color gradient from green (1960) to blue (1986). **D) Modern** (2007-2025, 18 years): herbarium records shown as dark purple circles; iNaturalist observations shown as light purple circles.

Dawson era records (1942-1959) included specimens collected by E. Yale Dawson (Figure 2.1 B, triangles) with the majority of the PVP records during this period made by Norman Cooper as part of the Allan Hancock Foundation. While there are just under half as many records during this period (n=36, noting it is a shorter duration of time), they still cover from Malibu (north LA County) down to Orange County, including both Santa Catalina Island and the PVP (Figure 2.1 B). However, there is a lack of observations in the south-eastern half of the PVP and in the Santa Monica Bay (Figure 2.1 B).

The Intermediate era period comprises herbaria records (1960-1986; Figure 2.1 C) that documented one observation in Malibu (Robert Setzer of the Allan Hancock Foundation), several in Laguna Beach and on Santa Catalina Island (various researchers), but zero from the Palos Verdes Peninsula region (Figure 2.1 C). There is a gap in any records accessed in this study from 1986-2007.

The Modern era (2007-2025; Figure 2.1 D) comprises both iNaturalist and herbaria records. Only three herbarium records were found between 2007-2018: one on San Clemente Island, one in Laguna Beach, and one in Palos Verdes near Flat Rock Point (Figure 2.1 D). With the addition of iNaturalist data, a total of 30 observations of *S. agardhianum* have been reported since 2007. Within these 30 observations, the vast majority were within Orange County Marine Protected Areas (MPAs), with only one on PVP (the Flat Rock Point herbaria record mentioned above).

Taken together, there was a marked decrease in recorded observations from the Palos Verdes Peninsula but a maintenance of observations from MPAs in Orange County when compared to either earlier period. However, it is important to note that

decreased observations may or may not be symptomatic of decreased presence/distribution over time.

Current Survey Indicates Stable *S. agardhianum* Populations in Orange County

Given the persistence of Orange County observations in herbaria and iNaturalist records, we hypothesized that there would be stable populations in Orange County. To assess the current presence of *S. agardhianum* in Orange County, we conducted field surveys at Corona del Mar, Crystal Cove, Shaws Cove, Goff Cove, and Dana Point (Figure 2.2 A, Appendix Table A2.2) from Dec. 2024-May 2025. *S. agardhianum* was not detected at Dana Point, likely due to unfavorable tidal conditions during the survey; recent iNaturalist observations suggest it may be present at this location. *S. agardhianum* was not found in Corona del Mar (Crystal Cove SCMA), adjacent to the Newport Harbor. *S. agardhianum* was found at the remaining three sites in lower intertidal wave-exposed habitats, often covering the peaks and ridges of rocky reef substrates among turf and coralline algae (Figure 2.2 A).

In these exposed habitats, it was often found in the intertidal layer above other California native Sargassaceae, *Stephanocystis dioica*. Other macrophytes such as *Laurencia spp., Corallina chilensis*, *Endocladia spp., Ulva spp., Colpomenia spp*., and various crustose species were also found co-occurring with *S. agardhianum*, along with *Phyllospadix torreyi* (seagrass) growing nearby. With variances in topography at Crystal Cove, *S. agardhianum* was additionally found blanketing lone boulders in the wave-exposed lower intertidal. Although the Orange County sites were informally observed to have high human visitation, *S. agardhianum* was found in areas that are difficult to access and may have had less traffic. The Crystal Cove population was the largest

observed in Orange County during our surveys (Figure 2.2 B). These results confirmed the presence of *S. agardhianum* at three of five surveyed Orange County sites, consistent with observations in this region spanning over 100 years, providing a contrast to the reduced frequency of observations recorded in the Palos Verdes Peninsula.

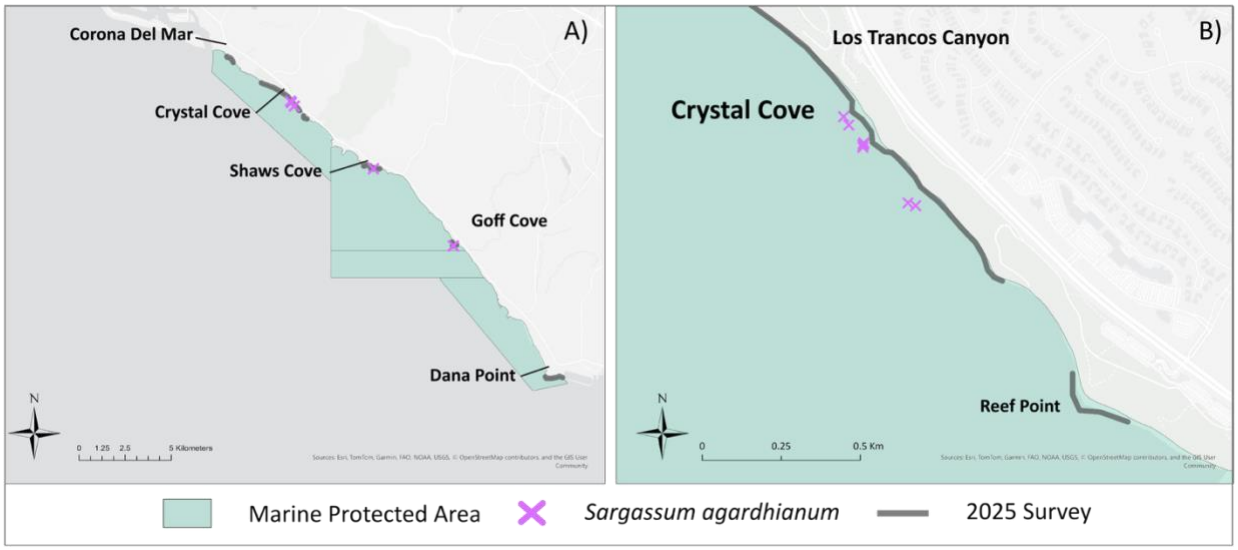

Figure 2.2

***Sargassum agardhianum* observations at Orange County survey sites (April 2025—April 2026).** Purple X markers indicate *S. agardhianum* observations; grey polylines indicate surveyed areas. **A)** Orange County survey sites; *S. agardhianum* was detected at Crystal Cove, Shaws Cove and Goff Cove but not at Dana Point or Corona del Mar. **B)** Extent of the *S. agardhianum* population at Crystal Cove, shown for size comparison with the Blufftop population (Figure 2.3 B).

Current Surveys Indicate a Single *S. agardhianum* Population Exists in Palos Verdes

Based on the decreased frequency of *S. agardhianum* observations over time in Palos Verdes Peninsula and the increased urbanization in LA County, we hypothesized that fewer populations would be detected compared to historical records. To investigate the state of *S. agardhianum* populations in the PVP, site surveys were conducted from April 2025-April 2026. The 10 survey sites were chosen to match the locations of Dawson's surveys (Dawson 1959, 1965) and sites of historical records (Figure 2.3 A;

Table 2.1); we were unable to survey the area around Abalone Cove and Portuguese Bend due to landslides prompting site access closures.

S. agardhianum was only observed at one site in the PVP (‘Blufftop’, Figure 2.3 B). This site is referred to as ‘Blufftop’ in this publication since it is accessed from the Blufftop trail. Details of our observations at Blufftop follow in the next section. *S. agardhianum* was not observed at any other survey site in the Palos Verdes Peninsula, including Flat Rock Point to the north where a 2018 herbarium record had been generated from (UCLA Mildred Mathias Herbarium; Figure 2.1 D).

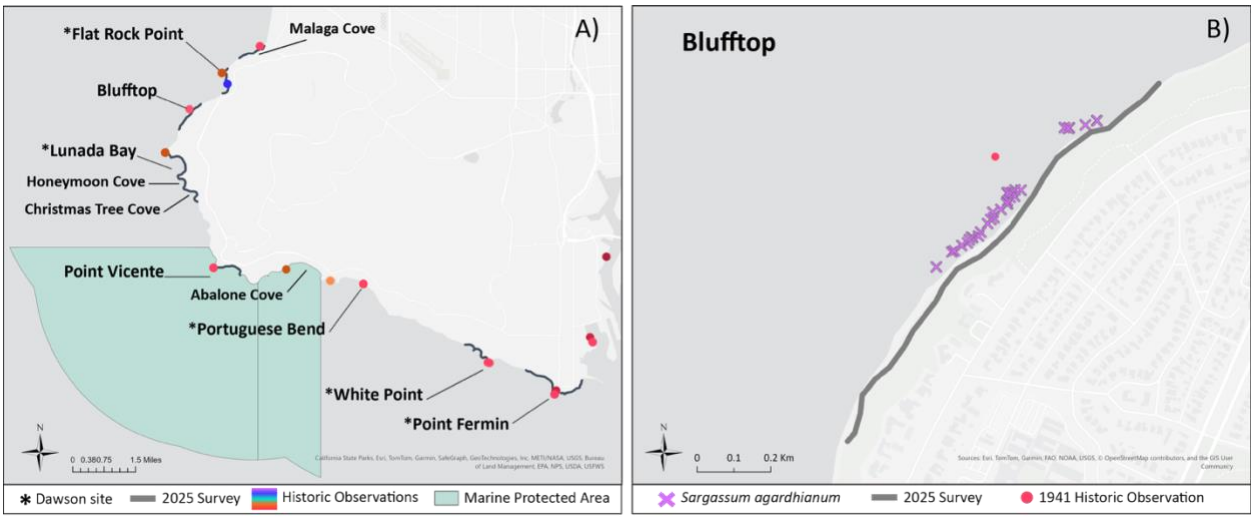

Figure 2.3

***Sargassum agardhianum* survey sites and observations on the Palos Verdes Peninsula (April 2025—April 2026).** Grey polylines indicate surveyed areas. **A)** Palos Verdes Peninsula survey sites with historical and modern observation records from all eras (see Figure 2.1 for era-specific colors), depicted as a color gradient; asterisks (*) denote Dawson survey sites. **B)** Extent of the *S. agardhianum* population at Blufftop; purple X markers indicate 2025 survey observations from this study and the single historical observation from 1941 is labeled on the map.

Community composition varied across PVP sites where *S. agardhianum* was absent. White Point was characterized by sparse macroalgae cover, dominated by crustose coralline algae, purple urchins, and anemones. Malaga Cove had a limited intertidal extent with sparse, low diverse macroalgal coverage of different composition

than White Point. At Flat Rock Point, Lunada Bay, Point Vicente, Point Fermin, and portions of Honeymoon cove and Christmastree Cove, macroalgal communities were observed with high cover and species composition similar to that found at Orange County sites where *S. agardhianum* was present, yet *S. agardhianum* was not detected at any of these locations.

The Portuguese Bend area and adjacent coves were inaccessible due to recent landslides prompting closures. *S. agardhianum* was not found along the western side of the protruding point at Point Fermin; as the eastern side was inaccessible, its presence cannot be excluded. Additional surveys were conducted on its historical distribution north of the Santa Monica Bay (Point Dume & Topanga Beach, Figure 2.1 AB) and *S. agardhianum* was not observed (data not shown). These observations, in addition to the lack of suitable rocky substrate in the Santa Monica Bay since the 1940s (Devienne 2024), suggest that the Blufftop population represents the current northern range limit of *S. agardhianum* and is the only known population on the mainland of LA County.

S. agardhianum Persists at Blufftop in Two Distinct Intertidal Habits

At Blufftop, the only Palos Verdes Peninsula population of *S. agardhianum* we found, had a range of approximately 1.1km of coastline, covering at least a 20 m wide swath of intertidal (Figure 2.3 B). Within this range, *S. agardhianum* was found in two habitats: the lower intertidal in areas with high wave exposure, observed in seaward facing areas (Figure 2.4 A; similar to Orange County sites), and in mid-to-high intertidal sheltered pools with minimal wave exposure where it co-occurred with *S. muticum* (Figure 2.4 B), consistent with historical descriptions of this species occupying both habitat types (Setchell and Gardner 1925). The composition of co-occurring macrophyte

communities at the exposed, lower intertidal habitats, mirrored the populations assessed in Orange County mentioned above.

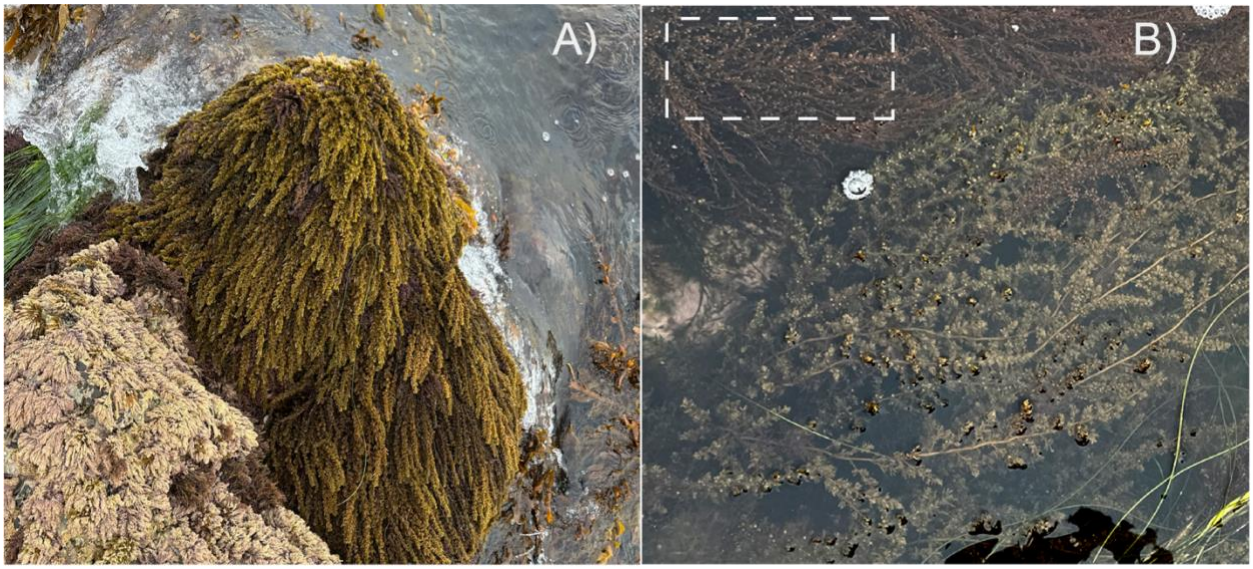

Figure 2.4

***Sargassum agardhianum* habitat and morphology at Blufftop. A)** *S. agardhianum* in the lower intertidal, wave-exposed habitat, blanketing a rock substrate during low tide. **B)** *S. agardhianum* submerged in a mid-to-high intertidal tidepool, displaying longer and thinner branch morphology, co-occurring with *Sargassum muticum* (dashed box).

Median branch length at Blufftop did not differ significantly from Crystal Cove (median difference = -1.23 cm, p=0.376; Figure 2.5) although Blufftop displayed a notably longer distribution tail with exceptionally long individuals observed at this site. Goff Cove and Shaws Cove both showed significantly reduced branch lengths relative to Crystal Cove (GC: -6.07 cm, p=0.031; SC: −9.46 cm, $p < 0.001$; Figure 2.5), consistent with field observations of comparatively sparse and patchy populations at those two sites. P-values were derived from a randomization test with Crystal Cove as the reference site.

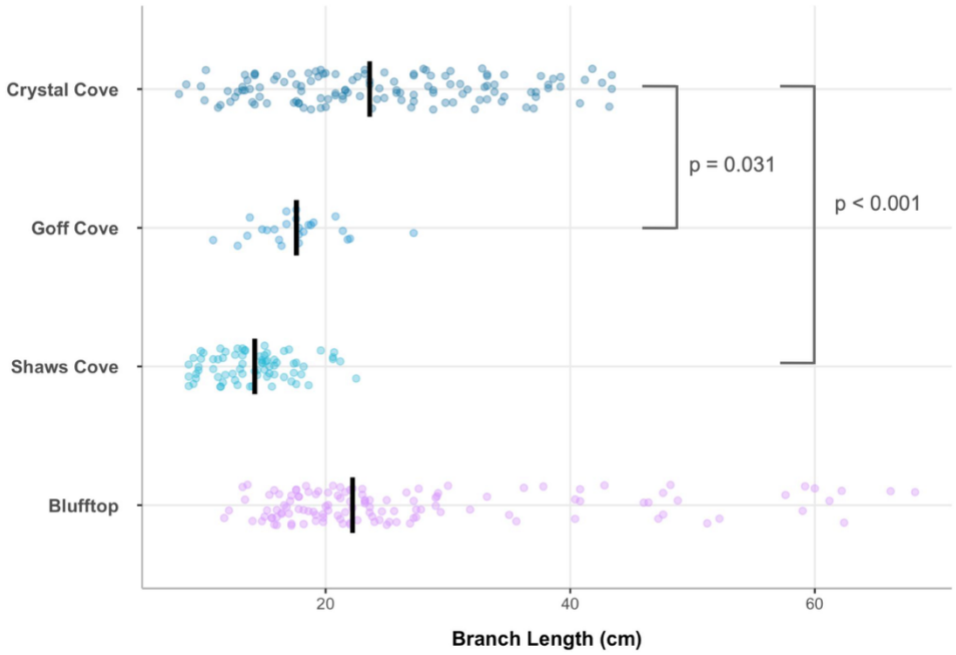

Figure 2.5

Comparison of *Sargassum agardhianum* branch length at Blufftop (Palos Verdes Peninsula) and Orange County sites, with Crystal Cove as the reference. Branch length (cm) of *Sargassum agardhianum* at four sites: Crystal Cove (n=125), Goff Cove (n=25), Shaws Cove (n=75), all in Orange County (reference site: Crystal Cove), and Blufftop (Palos Verdes Peninsula, n=125). Individual measurements are shown as colored points with median indicated by a vertical black bar. Significant P-values (<0.05) are indicated (grey bars and text to the right of data distributions) based on a randomization test comparing each site to Crystal Cove as the reference site.

Reproductive Phenology, Morphology, and Gamete Visualization of *S. agardhianum*

During our surveys, we documented the reproductive window of *S. agardhianum* and its reproductive structures. The literature states that *S. agardhianum* dies back during the winter and grows vegetatively in the late spring-summer (Miller 2024b). In the related Baja *Sargassums,* growth is followed by reproduction in the spring (McCourt 1984b). Based on comparing similar sea surface temperatures between the Baja range (Sicard-González et al. 2012) and Palos Verdes Peninsula, we hypothesized that the Blufftop *S. agardhianum* population would likely be reproductive in summer.

S. agardhianum was first identified at Blufftop at the end of June 2025, and at this time reproductive individuals were found in the mid-to-high intertidal pool populations but not the lower intertidal exposed patches. By the end of July, all *S. agardhianum* at Blufftop had identifiable reproductive branches and both habitats continued to do so through to the end of October 2025, and by late October/early November, receptacle axils were shed, indicating the end of the reproductive period (Figure 2.6).

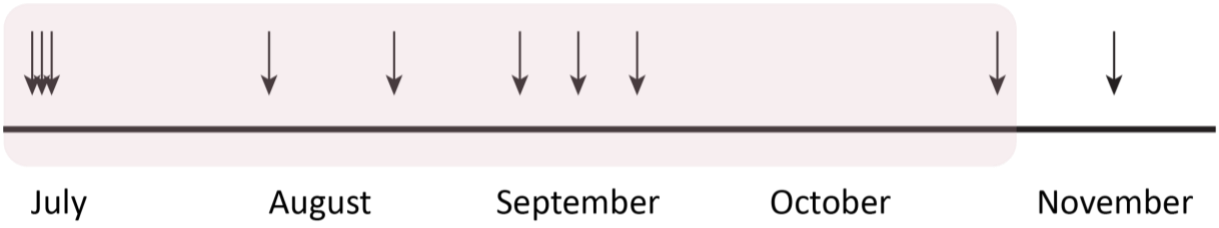

Figure 2.6

Reproductive timeline of *Sargassum agardhianum* at Blufftop. Arrows indicate survey dates at Blufftop. The pink box represents the period during which reproductive branches and receptacles were observed.

Consistent with literature on related Baja *Sargassums* (McCourt 1984b), the reproductive branches bearing receptacles were observed to be the longest compared to vegetative branches (Figure 2.7 A). Visually, reproductive branches of *S. agardhianum* had reduced blades and noticeably thick axial clumps (bearing receptacles) (Figure 2.7 BD). Receptacle morphology was observed to either be oblong and elongated or curved, with tooth-like projections along the apex and margins (Figure 2.7 BCD), consistent with historical descriptions (Agardh 1889, Setchell and Gardner 1925). In visits shortly after reproductive morphology was first observed, reproductive branches displayed large sub-apical naked stretches devoid of axial clumps/receptacles indicating that receptacles may have been shed (Figure 2.7 E); such naked stretches

were helpful in identifying reproductive branches in the field. Starting in July, reproductive branches became highly colonized by epiphytes at Blufftop, including *Ulva spp., Colpomenia spp.,* and red algae such as *Chondria acrorhizophora.* In many cases, a single red alga was overwhelmingly dominant such that it almost completely masked *S. agardhianum* from the eye (Figure 2.8); this red alga was likely *Pterocladiella capillacea* (Dr. Murray correspondence).

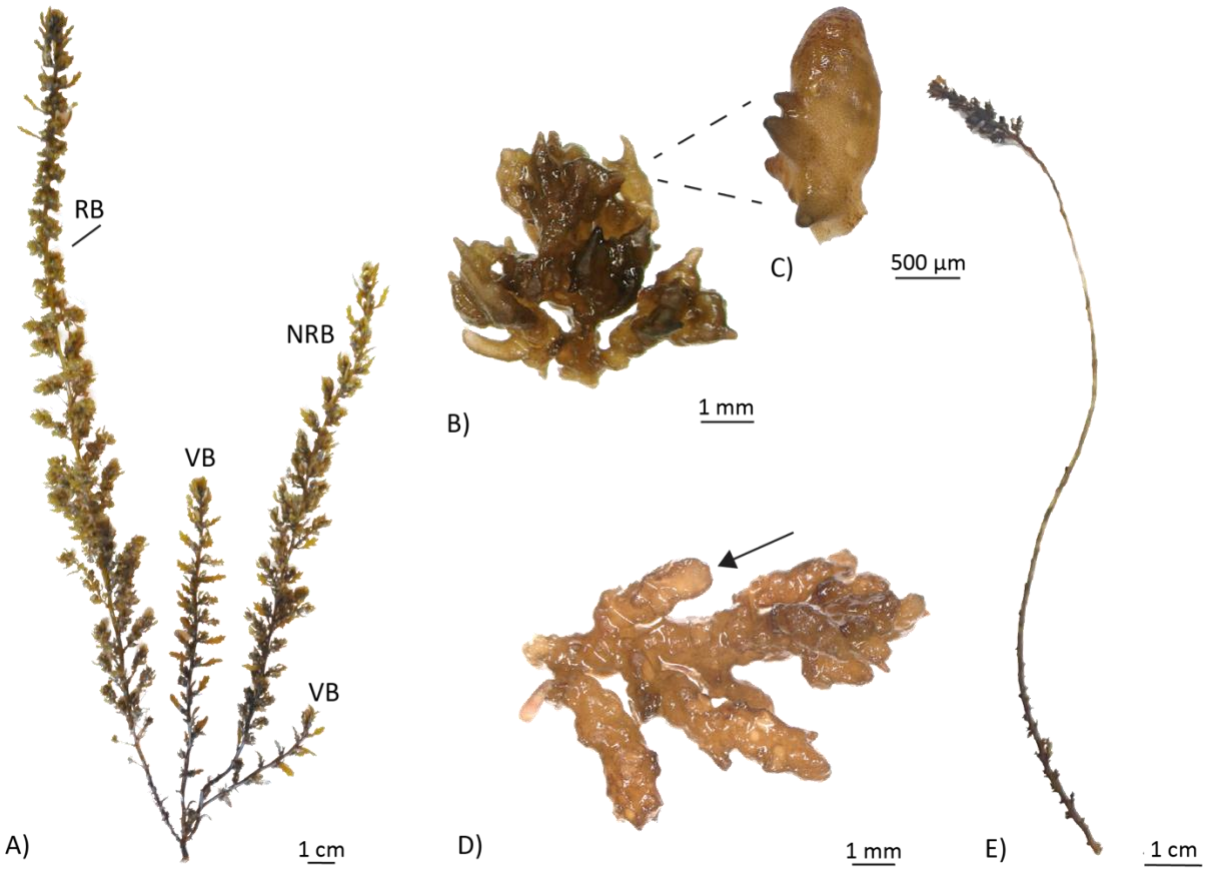

Figure 2.7

Reproductive morphology of *Sargassum agardhianum*. A) *S. agardhianum* thallus with vegetative branches (VB), a reproductive branch (RB), and a new reproductive branch (NRB). **B)** Reproductive axil bearing multiple receptacles with oblong morphology, with tooth-like projections along the margins. **C)** Isolated receptacle showing tooth-like projections along the margins. **D)** Elongated receptacle morphology on a reproductive axil (arrow). **E)** Reproductive branch following receptacle shedding, with only remnant receptacles remaining at the branch apex.

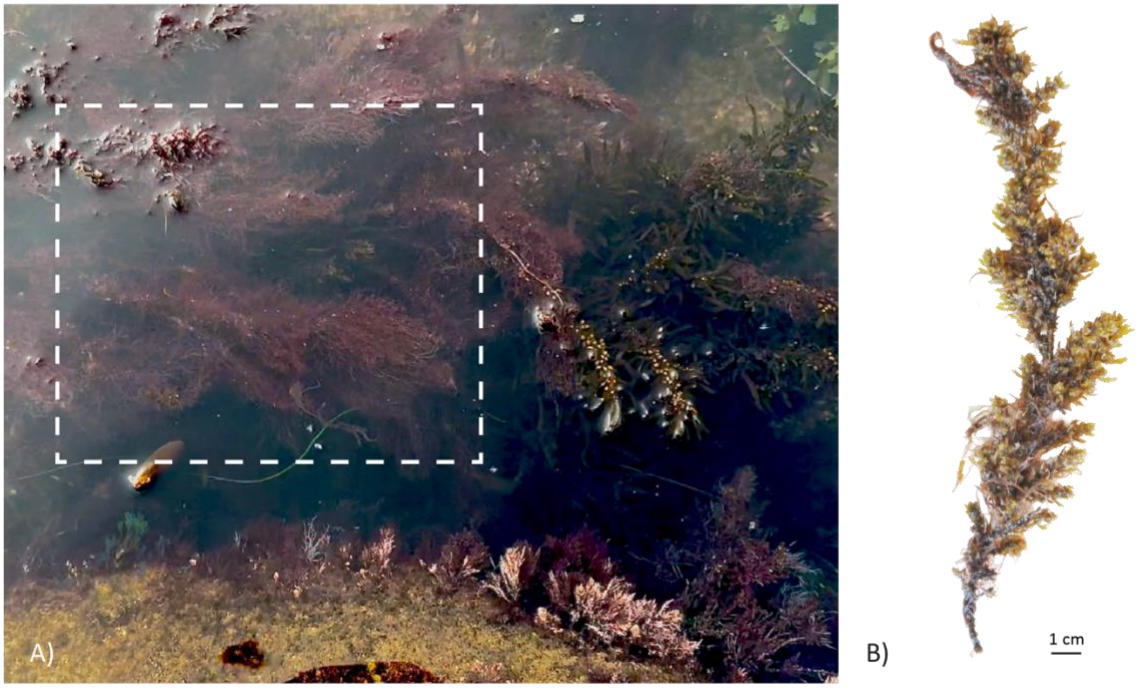

Figure 2.8

Red epiphyte on reproductive *Sargassum agardhianum.* A) *S. agardhianum* (dashed box) saturated in *Pterocladiella capillacea* epiphytes in a tidepool. **B)** Single reproductive branch with early *P. capillacea* epiphyte colonization.

Cross sections of the receptacles revealed some bearing only oogonia, some only antheridia, and some containing both in separate conceptacles, within the same individual (Figure 2.9). Thus, we conclude that *S. agardhianum* is monoecious with the receptacles sometimes only bearing one sex of the gametes, consistent with historical description (Setchell and Gardner 1925).

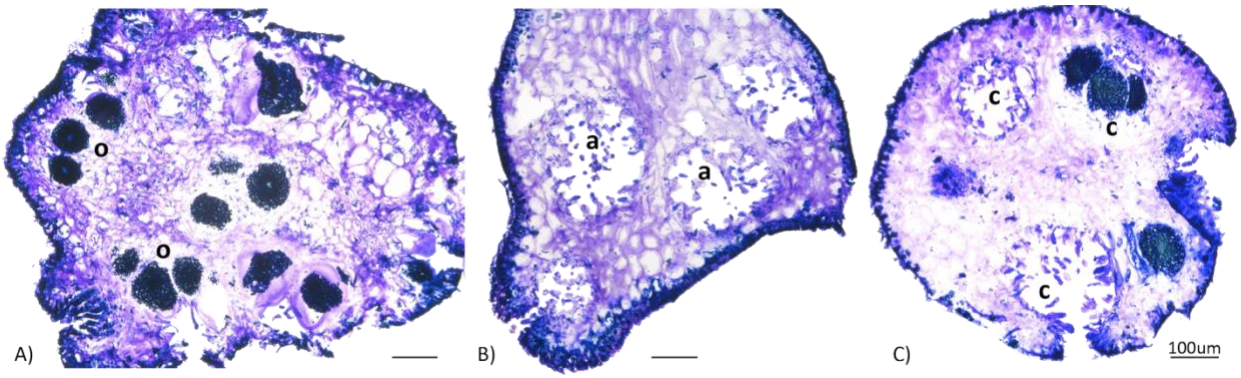

Figure 2.9

Cross sections of *Sargassum agardhianum* receptacles stained with Toluidine Blue O. A) Conceptacles containing oogonia only (o). **B)** Conceptacles containing antheridia only (a). **C)** Receptacle containing both antheridia and oogonia in separate conceptacles (c). Scale bars = **100 µm**.

DISCUSSION

Reproduction & Range Limitation

In agreement with previous descriptions (Miller 2024b), *S. agardhianum* elongated its branches in the late spring; however, the specific reproductive window following vegetative growth had not been previously described. Our surveys documented that reproductive branches developed by June (early summer) and receptacle axils were shed by late October/early November, establishing a summer-fall reproductive season for this species. Given that reproduction occurred across sea surface temperatures of 17C-22C (Appendix Table A2.1), and baseline ocean temperatures in the SCB are increasing at a rate of 0.12C per decade (Fumo et al. 2020), *S. agardhianum*'s summer-fall reproductive window may position this species to persist or increase in ecological importance as warming conditions become more prevalent in the region.

Over the course of the surveys, potential limitations to *S. agardhianum*'s reproductive strategy were observed. First, *S. agardhianum* was usually found in large, connected patches or blanketing a substrate (Figure 2.4 A). This habit may indicate that it recruits very locally, or perhaps self-fertilizes (Monteiro et al. 2009, Hughes et al. 2015). Secondly, there appears to be only 1-2 reproductive branches per individual, as opposed to each branch bearing receptacles. Third, there was variability in the number and presence of pneumatocysts on branches that may result in decreased raft/wrack dispersal of reproductive branches after detachment. As mentioned in Abbott and

Hollengberg (1976), some forms completely lack pneumatocysts. Lastly, it is possible that overgrowth of epiphytes (such as *P. capillacea*) may limit gamete dispersal. Interestingly, mere weeks after going reproductive, a branch would 'shed' the receptacles (Figure 2.7 E) similar to *S. muticum* (Baer and Stengel 2010b, Hughes et al. 2015), but it is unclear how this occurs nor its impact on reproduction and dispersal. Taken together, it appears that *S. agardhianum* may have a naturally small dispersal range.

Reduced Detection of *S. agardhianum* on the Palos Verdes Peninsula

Our observations detected *S. agardhianum* at far fewer locations on mainland Los Angeles County than historically recorded. Although not included formally here (Appendix Table A2.2), we note that we were unable to locate *S. agardhianum* at Point Dume (Malibu) where it had been observed in the Dawson and Intermediate era records. Given our extensive surveys along the Palos Verdes Peninsula, it is likely that the current detectable range of *S. agardhianum* on the mainland is more limited than historically recorded, but populations on Santa Catalina Island and Orange County appear to be stable. The single remaining population at Blufftop had not been formally recorded since 1941 by Allan Hancock Foundation researchers, representing an 84-year observation gap at this site.

Anthropogenic Stressors in the Palos Verdes Peninsula Region

Littler and Murray (1975) found that *S. agardhianum* would grow no closer than 10 meters to a sewage outfall on San Clemente Island. At this site, *S. agardhianum* and two other macroalgae and one seagrass species (*P. torreyi*) accounted for 35% of the intertidal coverage in unpolluted control plots but were conspicuously absent from the

outfall (Littler and Murray 1975). This documented sensitivity to sewage outfall pollution may help explain the reduced detection of *S. agardhianum* in the PVP, where outfall discharge has a long history. In contrast, populations in Orange County and Catalina Island, where outfall impacts are lower, remain detectable.

One hypothesis for the reduced detection of *S. agardhianum* throughout the PVP is a low recruitment and recovery rate following anthropogenic disturbances. Littler and Murray (1978) further investigated early algal succession at San Clemente Island, describing *S. agardhianum* as growing abundantly with 16.9% cover in unpolluted plots. In experimentally cleared plots, *S. agardhianum* had recovered to only 11.8% cover after 30 months (Murray and Littler 1978). Thus, it is hypothesized that *S. agardhianum* faces challenges with recovery after disturbance, potentially exacerbated through its reproductive strategy.

White Point was observed to be the least diverse site, consistent with its long history of ocean outfall discharge. The A.K. Warren Water Resource Facility (formerly JWPCP) has discharged treated wastewater offshore of White Point since 1937 (Rawn 1965), with full secondary treatment only achieved in 2003 (Kokesh et al. 2022). In 2010, the facility discharged an average daily flow of approximately 280 million gallons per day directly to the ocean (Highter and Allen 2012). Harris (1980) hypothesized that improvements observed at four of five PVP sites since Dawson's surveys may have resulted from the switch to deeper water outfalls in 1967, and the removal of solids from sewage systems starting in 1977.

The White Point outfalls also have a documented history of DDT and PCB contamination before they were banned in the 1970s. From 1947-1971 large quantities

of DDT were discharged from the Montrose Chemical plant in Los Angeles to the JWPCP and subsequently to the Palos Verdes Shelf (Palos Verdes Peninsula Watershed Management Group 2019). Although concentrations have decreased, DDT and PCBs persist in surface sediments at levels that can accumulate in fish tissues (Palos Verdes Peninsula Watershed Management Group 2019). The Palos Verdes shelf remains an active EPA Superfund site. DDT and PCBs have been shown to accumulate in related species of macroalgae (Pavoni et al. 2003), raising concerns for bioaccumulation in higher trophic levels (Grigg 1979, Pavoni et al. 2003, Qiu et al. 2017). However, these toxins have yet to be measured in PVP macroalgae and warrant future investigation.

The macroalgal communities at White Point were consistent with previously documented impacts and community shifts at this site (Widdowson 1971, Thom and Widdowson 1978, Harris 1983, Murray and Littler 1984, Gerrard 2005), with turf-forming and crustose species found in abundance, few large macroalgal species present, and notably sparse overall macroalgal cover. Purple urchin aggregations were observed across much of the intertidal at this site, which may further contribute to macroalgal suppression and recovery (Miller et al. 2022). Given the documented susceptibility of *S. agardhianum* to sewage outfall proximity (Littler and Murray 1975) and the long history of outfall discharge at White Point, combined with high human visitation (Murray and Littler 1984), the absence of *S. agardhianum* at this site is consistent with these compounding anthropogenic stressors (Harris 1983, Gerrard 2005).

Malaga Cove had a very small intertidal extent and was observed with sparse macroalgal cover and low overall diversity. These characteristics may reflect sand

deposition in the Santa Monica Bay, high human traffic (anecdotal observation), and the presence of freshwater input from Malaga Creek.

Flat Rock Point, Lunada Bay, Honeymoon Cove and Christmastree Cove supported macroalgal communities with higher diversity than White Point, with species composition in some areas resembling those observed at Orange County sites where *S. agardhianum* is present. Despite this, *S. agardhianum* was not detected at any of these locations, suggesting that factors beyond general community health may be limiting its presence, such as a historical disturbance from which it has not recovered, localized stressors, or natural variation in distribution.

At the north end of Lunada Bay the presence of shipwreck SS Dominator, along with associated rusting metal structures, was observed (Appendix Figure A2.1) and may represent an additional localized stressor to macrophyte communities in the surrounding area. Further investigation into the contamination effects of this wreck and local current patterns would help clarify its potential influence on surrounding macroalgal communities.

These western-facing sites (Flat Rock Point, Lunada Bay, Honeymoon Cove, Christmastree Cove) were observed to be relatively less impacted by the southern disturbances associated with the White Point outfall (Gerrard 2005), which may contribute to the more diverse communities observed there. However, the absence of *S. agardhianum* at these sites may also reflect its documented slow recovery rate following disturbance (Murray and Littler 1978), suggesting that even where conditions appear stable, populations may have not recovered from past impacts.

Point Vicente, within the Point Vicente State Marine Conservation Area, supported a relatively diverse macrophyte community, although its composition differed from that observed at Orange County sites. With only one record of *S. agardhianum* from 1931, its presence at this site may have been transient, and the differing community composition suggests local conditions may not have been optimal for *S. agardhianum*. As an accessible MPA, this site may experience elevated human visitation. Future studies could explore the dynamics shaping macroalgal communities here.

Point Fermin presented a mixed picture. The western-side of the point itself supported diverse macroalgal communities, resembling those observed at sites where *S. agardhianum* was present, yet *S. agardhianum* was not detected. The flanking beaches and intertidal areas showed evidence of high human disturbance including graffiti and debris. As the eastern side of the point was inaccessible, future studies should assess whether *S. agardhianum* is present there.

The Portuguese Bend area was unable to be surveyed due to landslide closures. Given the Palos Verdes Peninsula's known history of land movement and unstable bluffs, *S. agardhianum* populations in this area may be affected by scouring and burial (Pondella et al. 2018). Further research should assess this area once access is restored.

Table 2.1

***Sargassum agardhianum* records and field survey results at Palos Verdes Peninsula sites across four eras.** Flat Rock Point, Lunada Bay, Portuguese Bend, White Point, and Point Fermin represent Dawson's five Palos Verdes Peninsula survey sites **(Dawson 1959, 1965)**; remaining sites were selected based on herbarium and iNaturalist records. The 2025 survey column reflects the 10 field survey sites in this study.
"No record"- site was surveyed during that era but *S. agardhianum* was not documented.

"Not visited"- site was not surveyed by researchers during that era to the best of our knowledge; absence of record does not indicate absence of the species.
† Portuguese Bend not visited due to landslides and access closure.
‡ Modern era records are derived from herbarium records, iNaturalist observations, and Gerrard (2005), who surveyed the five Dawson sites but did not record *S. agardhianum* at any location.

Site name	Early era (1896-1941)	Dawson era (1942-1959)	Intermediate era (1960-1990)	Modern era (2000-2025)	2025 survey This publication
Flat Rock Point	Present	No record	No record	**Present**	Absent
Lunada Bay	No record	No record	No record	No record	Absent
Portuguese Bend	Present	No record	**Present**	No record	Not visited†
White Point	Present	No record	No record	No record	Absent
Point Fermin	Present	No record	No record	No record	Absent
Malaga Cove	Present	Not visited	Not visited	Not visited	Absent
Blufftop	Present	Not visited	Not visited	Not visited	**Present**
Honeymoon Cove	Not visited	Not visited	Not visited	Not visited	Absent
Christmastree Cove	Not visited	Not visited	Not visited	Not visited	Absent
Point Vicente	Present	Not visited	Not visited	Not visited	Absent

S. agardhianum Blufftop Population Characteristics

Of all our Palos Verdes Peninsula sites, *S. agardhianum* was only observed at Blufftop. Prior to this study, the last known observation of *S. agardhianum* at this site was in 1941 by Allan Hancock Foundation researchers. Strikingly, the Blufftop population was the largest we observed on the mainland, including those observed at Orange County sites (Figure 2.3 B vs Figure 2.2 B), and supported two distinct habitats for *S. agardhianum*.

The Blufftop site is accessed by a steep, seemingly unmaintained and eroding trail with minimal observations of other visitors over the course of recurring surveys. We hypothesize that the difficult trail access reduces human foot traffic and disturbance at this site, contributing to the persistence of *S. agardhianum*.

S. agardhianum at Blufftop was found in the mid-to-upper intertidal in deep tidepools, as well as wave-exposed rocks similar to those at Orange County sites. It was found with notably long branch lengths, including one individual reaching 87cm (not included in quadrat sampling), the longest recorded in this study. These pool subpopulations developed canopies that were observed co-occurring with *S. muticum*, suggesting potential competition for canopy space.

The morphological differences observed between pool and wave exposed *S. agardhianum* at Blufftop are consistent with historical descriptions of habitat-dependent form in this species (Setchell and Gardner 1925), and with patterns documented in related Baja *Sargassums* (McCourt 1984b). Although median primary branch length did not differ significantly between Blufftop and Crystal Cove, the Blufftop population displayed a notably longer distribution tail (Figure 2.5), with exceptionally long individuals observed at this site. Given that Blufftop uniquely supports both pool and wave-exposed populations, it is possible that the longer tail reflects contributions from pool-dwelling individuals, though branch length was not recorded by habitat type and this cannot be confirmed. Field observations at Blufftop suggest a distinct pattern in secondary branch morphology. Individuals found in deeper pools in the mid-to-upper intertidal at Blufftop exhibited noticeably elongated secondary branches, which was not observed in the wave-exposed lower intertidal. This extended secondary branching was absent at Crystal Cove, despite some individuals reaching comparable branch lengths. This suggests that overall branch length and secondary branch elongation may respond to the hydrodynamics or time spent submerged in the environment. Given their more

sheltered exposure to wave action, it is possible that deeper pools with elongated morphology may serve as a propagule refugium for the persistence of *S. agardhianum*.

The Blufftop site had a noticeably more challenging trail to access the coast and was rarely observed to have visitors. This site likely experiences fewer human disturbances than other accessible PVP sites. Among all the PVP sites, Blufftop appears to represent similar overall communities as OC County populations and conditions favorable for *S. agardhianum* to persist. We hypothesize *S. agardhianum*'s persistence at Blufftop reflects lower local anthropogenic pollution pressures and reduced foot traffic due to its difficult trail access.

Orange County and Catalina Island *S. agardhianum* Population Comparisons

All observed *Sargassum agardhianum* populations in Orange County were within Marine Protected Areas, though the protective effectiveness depends on enforcement and visitation levels (Murray et al. 1999). Beyond MPA boundaries, the watersheds surrounding these sites are substantially less urbanized than those in Los Angeles County (U.S. Census Bureau 2020a, 2020b), likely contributing to lower anthropogenic pressure on nearshore communities. Crystal Cove State Park is surrounded by extensive conservation land with minimal coastal development, as documented by parcel-level mapping of the region (Friends of Harbors, Beaches, and Parks 2012).

Corona del Mar, a frequently visited beach adjacent to Newport Harbor (Murray et al. 1999), was the only historically recorded site where *S. agardhianum* was not detected in our surveys. With no iNaturalist or other reported observations at this site since 1970, this suggests a prolonged absence. Given the high human visitation (Murray et al. 1999), adjacent harbor urbanization and pier development, we

hypothesize that *S. agardhianum* is absent from Corona del Mar due to compounding anthropogenic effects.

Orange County has an ocean outfall that extends five miles off of the Huntington Beach shores at a depth of 200 ft, downstream of the more urbanized portion of the county (Orange County Sanitation District 2026). In contrast to the JWPCP, Orange County receives a total average flow of 185 MGD, of which more than 170 MGD is recycled through its Groundwater Replenishment System (Orange County Sanitation District 2026), substantially reducing its ocean outfall discharge.

The persistence of *S. agardhianum* at most historically recorded Orange County sites is consistent with our hypothesis of lower overall anthropogenic pressure in this region. Although *S. agardhianum* was found at most historic sites, it was not found in the mid-to-high intertidal as is found at Blufftop. We hypothesize that this lack of mid-to-high intertidal zonation reflects high visitation frequency at these sites. Although within a MPA, these sites drive high visitation and have had little enforcement to reduce visitor disturbance (Murray et al. 1999). Since the OC County *S. agardhianum* populations sit at the interface between wave breaks and lower intertidal zones, as documented by GPS coordinates recorded during field surveys (ArcGIS Field Maps), this can deter human visitation as there are other easily accessible zones. Thus, *S. agardhianum* may have historically occupied mid-to-high intertidal pools at these sites and may now be absent due to anthropogenic foot traffic pressure.

Field observations at Santa Catalina Island, supported by extensive historical and current observation records distributed across the island (Figure 2.1), suggest it supports the largest *S. agardhianum* population within the study region, though a

systematic survey has not been conducted. Santa Catalina Island is at an advantage being geographically isolated from the major urbanization of the mainland, with very minimal development. Although the island has many protected areas, *S. agardhianum* was easily found well outside of those borders in abundance. Taken together, the persistence of *S. agardhianum* in Orange County and Santa Catalina Island likely reflects a combination of reduced anthropogenic stressors including lower ocean discharge volumes, MPA protections, less urbanized watersheds, and greater geographic isolation, contrasting with the conditions hypothesized to its reduced detection in the PVP.

S. agardhianum has persisted in areas where outfall and urbanization impacts are lower (OC County) and where foot traffic is lower (Blufftop and OC County wave-exposed zones). We hypothesize that the combination of lower urbanization-driven pollution and reduced foot traffic provides favorable conditions for *S. agardhianum* persistence. Ongoing upgrades to the White Point outfall system (Highter and Allen 2012) may improve conditions for macrophyte communities, however, given its slow recovery (Littler and Murray 1975), *S. agardhianum* may not come back quickly, if at all. Therefore, continued monitoring of PVP macrophyte communities, alongside consideration of foot traffic management at accessible sites, will be essential to understanding whether and how *S. agardhianum* may recover.

S. agardhianum as a Candidate for an Indicator Species

The macroalgal communities where *S. agardhianum* was thriving in Blufftop mirrored the Orange County ecological community: *Laurencia spp., Corallina chilensis*, *Endocladia spp., Colpomenia spp., S. dioica,* various crustose species, and seagrass.

These observations position *S. agardhianum* as a possible indicator of intertidal ecosystem health. Conversely, it may also be a useful indicator of threatened ecosystems: several sites in the Palos Verdes Peninsula without *S. agardhianum* exhibited similar macroalgal communities indicating that *S. agardhianum* may be more sensitive to change than co-occurring species. The presence of *S. agardhianum* at Blufftop, the only PVP site where it was detected, may reflect relatively lower anthropogenic stress at this location compared to other PVP sites.

Littler and Murray (1975) documented that *S. agardhianum* was conspicuously absent near the San Clemente sewage outfall, where it was replaced by early successional and disturbance-tolerant species including crustose, filamentous, and small turf algae. This indicator pattern is supported by their subsequent work at White Point (Murray and Littler 1984), where communities were dominated by small, crustose, and delicate forms with thick leathery species comprising less than 1% of cover. This pattern was broadly consistent with our observations at White Point, suggesting that community composition may serve as a useful field indicator of ecosystem health.

Taken together, these observations identify *S. agardhianum* as a candidate indicator species for indicator ecosystem health; however, establishing it formally as an indicator species would require additional targeted studies.

Recently Introduced Comparison to *Sargassum agardhianum*

Sargassum muticum was first reported in the Palos Verdes Peninsula region by Thom & Widdowson (1978) and reported a low abundance at four out of five sites by Gerrard during their spring 2000 surveys (Thom and Widdowson 1978, Gerrard 2005). During our surveys, *S. muticum* was widespread at every site that was surveyed,

dominating across intertidal zones, and was found growing on both rocky substrates and sandy substrate. Although Stewart (1991) mentions *S. agardhianum* can be occasionally found in sand, this was not observed throughout this study. *S. muticum* was observed to easily reach large thalli lengths and create canopies wherever it grows. *S. muticum*'s reproductive season is highly variable during winter, spring and summer months (Smith 2016). Previous studies have demonstrated *S. muticum* to compete with native species and affect community structure in British Columbia, but its effects on native macrophyte communities in the PVP have yet to be determined (Ambrose and Nelson 1982, Wilson 2001). Future studies should investigate whether *S. muticum*, now observed ubiquitously across intertidal sites in both LA and Orange Counties, is competing with or displacing *S. agardhianum* and contributing to its reduced detection in the PVP, and systematic quantification of its current cover is warranted.

Why Does it Matter? Biodiversity & Climate Change

Littler & Murray (1974) found *S. agardhianum* to be among the top 10 primary producers in unpolluted intertidal communities at San Clemente Island, suggesting it plays an important role in coastal productivity. Other related *Sargassums* have been known to form habitats for a diverse array of invertebrates, fishes and epiphytic and understory algae (Aris et al. 1982, Bertness et al. 1999, Bulleri et al. 2002, Sapper and Murray 2003, Burnaford 2004, Whitaker et al. 2010, McCourt 1984b). In previous studies of herbivory choices between native and introduced seaweed species, no *Sargassum* (*S. agardhianum, S. muticum, S. horneri*) was preferred to be consumed, with only a slight preference to the native *S. agardhianum* in one study (Vogt 2011), and a slight preference for *S. muticum* in another (Kaplanis et al. 2020). Thus, it is

hypothesized that *S. agardhianum* is not a common native food source and is an important architectural species, serving as a habitat to invertebrates and epiphytic algae. This hypothesis is supported by observations made in situ and under a microscope. In contrast, *S. muticum* was not observed to have epiphytes or other epibiota throughout the yearlong surveys.

The most common abiotic stressors to intertidal communities are heat and desiccation, which can be relieved with macroalgal canopies (Bertness et al. 1999, Bulleri et al. 2002, Sapper and Murray 2003, Gerrard 2005, Whitaker et al. 2024). Continued decreases in canopy-forming macrophytes could impact other species that use macroalgae for shelter, refuge from predation, or as a nursery ground (Aris et al. 1982, Sapper and Murray 2003, Burnaford 2004, Gerrard 2005). The survival and growth of understory organisms or those that occur as epiphytes are affected by the presence of macroalgal canopies (Brown et al. 1990, Bertness et al. 1999, Bulleri et al. 2002, Sapper and Murray 2003, Gerrard 2005, Whitaker et al. 2024). Loss of canopy-forming species may amplify the presence of early successional species mentioned previously, which may lead to a decrease in community primary productivity. Since *S. agardhianum* is known to be a relatively productive primary producer (Littler and Murray 1974) and a potentially important habitat, further assessment of these remaining populations is warranted.

Gerrard (2005) hypothesized an increase in warm-water seaweed species at PVP sites coinciding with increases in regional water temperatures; however, no evidence of this was found despite surveys performed at the end of a 20-year warming period. Notably, *Stephanocystis dioica*, the only Sargassaceae historically noted as

common to abundant at these sites, was not recorded at any of the five PVP locations by (Gerrard 2005) yet was observed in abundance across all surveyed PVP sites during this study.

As of this publication, the Blufftop population resides at the highest latitude compared to other regional populations. With the ever-growing concerns of climate change and more frequent marine heatwave events (Fumo et al. 2020), understanding and preserving species known to tolerate warmer waters may prove valuable as latitudinal shifts are projected to continue. Range-edge environments are more susceptible to climatic events with the leading range edge populations acting as important steppingstones or by preserving unique genetic adaptations (Rehm et al. 2015). The Orange County and Santa Catalina Island populations were recorded to have warm waters during the growing and reproductive seasons, with sea surface temperatures exceeding 21°C during the reproductive season (Appendix Table A2.1). *Sargassums* are most commonly found in tropical and subtropical oceans (Stiger-Pouvreau et al. 2023), with *S. agardhianum* restricted to the warm-temperate waters of the SCB (Abbott and Hollenberg 1976). Given its thermal affinity to the Baja *Sargassums, S. agardhianum* may be a candidate indicator species for healthy warm-temperate intertidal communities. With projected increasing temperatures, northward range shifts may increase the ecological importance of the Blufftop population as a leading-edge refuge for this species.

Future Recommendations

The ecological relationships between *S. agardhianum* and the biodiversity it supports, including epiphytes and associated fauna, should be investigated. Identifying

whether the epibiota is using *S. agardhianum* as a habitat, refuge or nursery ground would illuminate the potential importance of our only native mainland *Sargassum*. Exploring the relationship between *S. agardhianum* and the epiphytic *P. capillacea* would help clarify whether this relationship is beneficial or harmful to the reproductive strategy. In addition, determining if there is competition between *S. agardhianum* and *S. muticum* would be valuable as its widespread abundance was conspicuous.

With the large gap of available genetic sequences, classic conservation genomics tools, (e.g., allele frequencies, F-statistics) would be useful to assess the status of genetic diversity and structure within these remaining populations. We recommend assessing Blufftop, since it is currently the only known population in this study to reflect diverse reproductive morphology and habitat zonation. We also recommend assessing whether selfing is occurring in all populations mentioned in this study, as this may be responsible for the homogenous patches of *S. agardhianum* observed. With existing genomic DNA extracted from multiple individuals of Blufftop, Crystal Cove, and Catalina, we recommend a pool-seq analysis, since it is cost-effective at comparing between population samples genome-wide (Kofler et al. 2011).

Lastly, future studies should further investigate the formation of conceptacles in relation to order of development compared to the type of gametes found within. Determining whether a hierarchical order exists in the position of sex-specific gametes along the individual would provide valuable insight. This information would be beneficial for potential future cultivation experiments, should *S. agardhianum* be part of a restoration effort. Although this study substantially advances our understanding of *S.*

agardhianum, many opportunities remain to further investigate this species and clarify its ecological role in Southern California's warm-temperate intertidal ecosystems.

APPENDIX 2

Figure A2.1

SS Dominator shipwreck and associated rusting metal structures observed in the intertidal zone at Lunada Bay, Palos Verdes Peninsula.

Table A2.1

Palos Verdes Peninsula survey site conditions, Sargassaceae species observations, and habitat characteristics.

Location	Date	Air Temp °F	Tide height (ft)	Water temp °F	Species	Habitat
Malaga Cove (33.803790, -118.396765)						
	07/16/2025	67	1.01	66.2	SC, SM	high-lower intertidal
Flat Rock Point / Bluff Cove (33.79355, -118.4068833)						

	12/01/2024	67	-0.6	59	SM, SC	high-low intertidal
	07/16/2025	66	0.56	66.2	SD, SM	lower intertidal/upper subtidal
	01/17/2026	72	-0.88	61.6	SO, SC, SH, SM	lower intertidal/upper subtidal
	01/19/2025	69	0	61.9	SM, SH	lower intertidal/upper subtidal
Blufftop (33.78628, -118.42026)						
	06/28/2025	64	-0.96	72	SA, SD, SM	lower intertidal/upper subtidal
	06/29/2025	73	4.01	72	SD, SM	lower intertidal/upper subtidal
	06/30/2025	63	0.12	71.6	SA, SD, SM	lower intertidal
	07/26/2025	65	-0.59	67	SA, SM	lower intertidal
	08/10/2025	65	-0.71	68.5	SA, SD, SM	lower intertidal/upper subtidal
	08/25/2025	68	0.3	71.8	SA, SM	lower intertidal/upper subtidal
	09/01/2025	81	4.25	73.3	SA, SM	lower intertidal/upper subtidal
	09/08/2025	74	5.51	71.6	SA, SM	lower intertidal/upper subtidal
	10/21/2025	68	0.14	66	SA, SD, SM	lower intertidal/upper subtidal
	11/4/2025	66	-0.61	65.5	SA, SD, SM	lower intertidal/upper subtidal
Lunada Bay (33.76876667, -118.4216667)						
	07/13/2025	62	-0.72	64.7	SD, SM	lower intertidal/upper subtidal
	07/14/2025	72	4.28	64.7	SO, SD, SM	upper subtidal

	07/15/2025	64	0.02	64.7	SD, SM	lower intertidal/upper subtidal
	08/10/2025	66	4.61	69.8	SO, SD, SM	upper subtidal
	10/12/2025	73	5.62	62.6	SD, SM	lower intertidal/upper subtidal
Honeymoon Cove (33.765538, -118.421661)						
	08/11/2025	64	-0.42	68.5	SD, SM	lower intertidal/upper subtidal
Christmastree Cove (33.762297, -118.417917)						
	08/12/2025	66	0.04	68.5	SD, SM	lower intertidal/upper subtidal
	08/28/2025	69	1.88	71.6	SD, SM	lower intertidal/upper subtidal
Point Vicente (33.741213, -118.407205)						
	12/30/2025	69	-0.37	62.2	SD, SO, SC, SM	lower intertidal/upper subtidal
White Point (33.7158, -118.32001667)						
	12/13/2024	62F	-1.28	59F	SM, SD	low-mid intertidal
	04/06/2025	77F	-0.31	53.6F	SO, SD, SM	upper subtidal
	07/10/2025				SM	upper subtidal
Point Fermin (33.7074, -118.28605)						
	07/29/2025	63F	0.66	65.7F	SD, SC	lower intertidal/upper subtidal
	09/06/2025	74F	2.01	69.6F	SD, SC	lower intertidal/upper subtidal
	09/18/2025	74F	1.89	70.3F	SD, SC	lower intertidal/upper subtidal

	10/04/2025	72F	1.38	67.5F	SD, SC	lower intertidal/upper subtidal
Species name:	SA - *Sargassum agardhianum*		SD - *Stephanocystis dioica*		SM - *Sargassum muticum*	
	SH - *Sargassum horneri*		SP - *Sargassum palmeri*		SC - *Silvetia compressa*	

Table A2.2

Orange County and northern Los Angeles County survey site conditions, Sargassaceae species observations, and habitat characteristics.

Location	Date	Air Temp °F	Tide height (ft)	Water temp °F	Species	Habitat surveyed
Shaw's Cove (33.5429, -117.7984)						
	12/14/2024	65	-1.55	60	SA, SC, SM	lower intertidal
	02/22/2025	73	1	60.8	SA, SD, SC	lower intertidal
	07/09/2025	73	3.53	62.6	SA, SD	lower intertidal/upper subtidal
	07/27/2025	66	-0.39	64.4	SA, SD	lower intertidal/upper subtidal
	10/05/2025	63	5.41	66.2	SA	lower intertidal/upper subtidal
Goff Cove (33.51369, -117.76027)						
	04/20/2025	66	0.3	60.8	SC, SA, SD, SM	lower intertidal/upper subtidal
	07/09/2025	77	2.5	64.5	SC, SA, SD, SM	lower intertidal/upper subtidal
	07/27/2025	66	-0.39	64.4	SC, SA, SD, SM	lower intertidal
	10/05/2025	66	5.01	67.2	SA	lower intertidal/upper subtidal
Crystal Cove (33.57193, -117.83920)						

	05/17/2025	61	-0.11	62.2	SA	lower intertidal/upper subtidal
	07/28/2025	66	0.13	63.7	SA	lower intertidal/upper subtidal
	08/26/2025	72	4.96	73.4	SA	lower intertidal/upper subtidal
	10/05/2025	72	0.63	67	SA	lower intertidal/upper subtidal
	10/19/2025	79	0.51	63.7	SA	lower intertidal/upper subtidal
	01/18/2026	74	-0.4	61.3	SA, SM	lower intertidal/upper subtidal
Dana Point (33.459983, -117.713304)						
	05/17/2025	65	0.75	62.2	SO, SM, SC, SD	lower intertidal
Corona del Mar (33.588828, -117.868239)						
	01/18/2025	74	-0.94	61.5	SO, SC, SM	lower intertidal
Descanso Bay (33.353709, -118.329702)						
	11/16/2025	54	4.01	64.4	SA, SD, SP, SC, PC, SH	lower intertidal/upper subtidal
Point Dume (34.003509, -118.804189)						
	12/29/2025	67	0.53	62.8	SC	
Topanga Beach (34.03726, -118.5822)						
	03/16/2026	74	0.1	62.4	N/A	lower intertidal
Species name:	SA - *Sargassum agardhianum*		SD - *Stephanocystis dioica*		SM - *Sargassum muticum*	
	SH - *Sargassum horneri*		SP - *Sargassum palmeri*		SC - *Silvetia compressa*	

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