

NEREOCYSTIS LUETKEANA (BULL KELP) IN SOUTH PUGET SOUND:
STRESSOR IMPACTS ON THE HEALTH OF NATIVE FLOATING KELP
CANOPIES

by

Maxwell D. Calloway

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Environmental Studies
May 2019

© Maxwell D. Calloway. All rights reserved.

This Thesis for the Master of Environmental Studies Degree

by

Maxwell D. Calloway

has been approved for

The Evergreen State College

by

Erin Martin

Member of the Faculty

[Date]

ABSTRACT

Nereocystis luetkeana (bull kelp) in South Puget Sound: Stressor Impacts on the Health of Native Floating Kelp Canopies

Maxwell D. Calloway

Bull kelp (*Nereocystis luetkeana*), is a critical, habitat-forming, floating seaweed found along Salish Sea's shorelines. East of the Strait of Juan de Fuca, *Nereocystis* is the sole floating canopy forming seaweed. Unfortunately, its abundance in Puget Sound is declining for unknown reasons. Little research exists on *Nereocystis* within Puget Sound, an inland sea with distinct environmental conditions and stressors from outer coast environments. In addition, stressors often interact synergistically in marine environments, compounding negative impacts more than would be predicted from single stressors alone. To assess the causes of decline in the Puget Sound, this study monitored *Nereocystis* blade length and plant density at four sites spanning a north to south gradient within the South Puget Sound. In addition, several common Puget Sound stressors were assessed including temperature, nitrate availability and densities of the native kelp crab, *Pugettia producta*. Generally, stress intensity followed a geographic gradient, increasing in the more interior waters of the South Puget Sound. High temperatures and crab densities were correlated with significant declines in blade length although no significant interaction between the two was observed. In addition, the bed exposed to the highest temperatures and crab densities was characterized by significantly lower densities of plants than any other bed selected for density monitoring. This study also documented the loss of a surface canopy in the South Puget Sound. Given that ocean temperatures are predicted to continue to rise over the next decades, it is likely that Puget Sound *Nereocystis* forests will continue to decline unless conservation and recovery actions are implemented.

TABLE OF CONTENTS

<i>Introduction</i>	1
<i>Literature Review</i>	3
Puget Sound: Place and Processes	4
Nereocystis lifecycle	6
Abiotic controls: Environmental conditions and physical forces	9
<i>Photosynthetic performance</i>	9
<i>Temperature</i>	10
<i>Nutrients</i>	12
Biotic controls in the SPS	13
Washington State kelp	15
<i>Methods</i>	18
Site Selection	18
Mean Water Column Temperature	21
Nitrate availability	22
Bed-wide and <i>Nereocystis</i> blade length monitoring	22
Analysis	23
<i>Results</i>	25
Survey Timing	25
Site differences	27
<i>Plant Density</i>	27
<i>Blade Length</i>	29
<i>Temperature</i>	32
<i>Pugettia producta densities</i>	35
<i>Nitrate Concentrations</i>	37
Interactions Between Temperature, Crab Density and Blade Length	39
<i>Discussion</i>	43
Plant and bed condition	43
Environmental and biotic stressors	44
Devil’s Head, substrate availability and bed area	47
Methods Assessment	48
Continued monitoring	49
<i>Conclusion</i>	49
<i>References</i>	51
<i>Appendix</i>	57

LIST OF FIGURES

Figure 1: Map of the Puget Sound showing major basins (Gelfenbaum et al. 2006).....	5
Figure 2: South Puget Sound and study sites.....	20
Figure 3: Site monitoring design.....	21
Figure 4. Plant densities at each site (black line = median, white diamond = mean).	27
Figure 5. Monthly plant densities at each site (mean \pm SE).	29
Figure 6: Blade length at each site (black line = median, white diamond = mean).....	30
Figure 7: Average monthly blade lengths of <i>Nereocystis</i> individuals at each site (mean + SE).....	31
Figure 8: Average monthly integrated water column temperatures.....	33
Figure 9: Crab densities at each site (black line = median, pink diamond = mean)	36
Figure 10: Average monthly crab densities at each site (mean \pm SE).	36
Figure 11: Growing season (May–September) water nitrate concentrations at study sites (black line = median, white diamond = mean).	38
Figure 12: Monthly growing season nitrate concentrations (μ M) at two depths (0.25 m and 4 m) at study sites.....	38
Figure 13: Blade length as a function of water temperature with regression trend line ...	40
Figure 14: Blade length as a function of crab densities with regression trend line	40
Figure 15: Squaxin Island blade length as a function of temperature with trend line of predicted values from regression analysis.	42

LIST OF TABLES

Table 1. Plant density per square meter as a function of site: Sample size, mean, standard deviation, standard error, median, first quartile, third quartile and interquartile range.	27
Table 2. Blade length (m) as a function of site: Sample size, mean, standard deviation, standard error and 95% confidence interval for.....	30
Table 3. Temperatures ($^{\circ}$ C) as a function of survey month and site with Games-Howell pairwise post hoc differences*: Sample size, mean, standard deviation and maximum integrated water column.....	34
Table 4. Crab densities as a function of site with Games-Howell pairwise post hoc test p-values: Sample size, mean, standard deviation, standard error.....	35
Table 5. Nitrate concentrations as a function of site: Sample size, mean standard deviation, standard error and 95% confidence interval	37

Table 6. Nereocystis blade length, temperature and crab densities at all sites: Multiple linear-regression with quadratic polynomial treatment of temperature, and interaction between crab densities and average temperature	39
Table 7. Nereocystis blade length, temperature and crab densities at Squaxin Island: Multiple linear-regression with quadratic polynomial treatment of temperature, and interaction between crab densities and average temperature	42
Table 8. Plant density per square meter as a function of site and survey date: sample size, mean, standard deviation, standard error, median, first quartile, third quartile and interquartile range of.....	57
Table 9. Blade length (m) as a function of site and survey month: sample size, mean, standard deviation, standard error, median and 95% confidence interval	58
Table 10. Crab densities as a function of site and survey month: sample size, mean, standard deviation, standard error, median, first quartile, third quartile and interquartile range.....	59
Table 11. Nitrate concentrations (μM) as a function of survey month and depth at each site: sample size, mean, standard deviation, standard error and 95% confidence interval	60

ACKNOWLEDGMENTS

This project owes its success to the absolutely amazing network of mentors, friends and peers in Olympia. First and foremost, I would like to thank my advisory committee Dr. Erin Martin of the Evergreen State College, Helen Berry of the Washington Department of Natural Resources Submerged Aquatic Vegetation program and Dr. Tom Mumford (former WA-DNR). I am indebted to their expertise, guidance and trust in my ability to complete a field-based project with a shoestring budget, bubble-gum and scotch tape. I owe special thanks to Helen Berry whose collaboration made the field component of this project possible. Thanks also to John Withey, and Bart Christiaen and Pete Dowty of WA-DNR for their willingness to brainstorm on statistical methods. Thank you to Erin Martin for believing that a scruffy dirt-bag with a degree in literature could make the transition to a career in science and for being patient as the writing component lasted longer than expected. And, of course, thank you to my wonderful Olympia family: Wolf and Rainier Mithrandir, and Tasa Anderson for reminding me to always look at the world with the curiosity and wonder of a child; John and Alexandria Messina for letting me steal their kayak for extended periods of time with no expectation that it, or I would ever come back but always suggesting we cook dinner, relax and enjoy house-plants on a front porch on summer evenings when I did; John Messina, Averi Azar, Keegan Michael Curry, Kirsten Miller, Julian Wischniewksi and Simon Young for their willingness to let me whisk them to kelp beds in a tiny dinghy with a small motor with a bad habit of over-heating. Thank you also to Kyle Grosten for volunteering his boat and time for field support. Finally, special thanks to the kelp forests at Salt Creek State Park for inspiring me to study kelp in the first place and Dr. Tim Quinn of the Washington Department of Fish and Wildlife for confirming that this whole crazy adventure was a good idea in the first place.

INTRODUCTION

The bull kelp, *Nereocystis luetkeana* (Mertens) Postels & Ruprecht (hereafter *Nereocystis*) is a monotypic, annual, kelp (order Phaeophyceae, class Laminariales) found along the temperate, eastern Pacific coast from southern California to Alaska (Druehl & Clarkston 2016). *Nereocystis*, along with the perennial giant kelp *Macrocystis pyrifera* (hereafter *Macrocystis*), form dense floating canopies throughout rocky, subtidal environments in the Pacific Northwest (PNW). Mixed canopies in Washington state occur along the outer coast and into the Strait of Juan de Fuca, but *Nereocystis* is the only floating canopy forming species found in the Puget Sound (Berry 2017).

Anecdotal accounts from local residents, tribes and management agencies suggest that *Nereocystis* canopies in the South Puget Sound (SPS) – the southernmost basin of the Puget Sound incorporating all waters south of the Tacoma Narrows (Fig. 1) – are declining in abundance and linear extent. These accounts have been confirmed by recent analyses of historical survey data conducted by the Washington Department of Natural Resources (WA–DNR) that document a 67 percent decrease in *Nereocystis* canopy linear extent from 1873 to 2017 (Berry 2018). In light of these considerable losses of SPS *Nereocystis* canopies, understanding causes for observed declines is paramount if conservation and restoration measures are to be successfully implemented. Unfortunately, no data exists on the response of Puget Sound bull kelp to common stressors such as temperature, nutrient availability and grazing pressure – three parameters known to significantly effect kelp populations elsewhere in North America and abroad (Steneck et al. 2002).

Assessing the impacts of stressors on kelp can be difficult as the majority of kelp species live out their entire lives in subtidal waters, requiring the use of SCUBA surveys

(Gabrielson et al. 2012). Even surveys of *Macrocystis* often require subtidal observations as all specialized reproductive blades (sporophylls) occur at the base of the plant (Schiel & Foster 2015). *Nereocystis* is unique in that it produces all of its reproductive tissue along vegetative blades that grow only at the surface, making surface assessments of stressor impacts comparatively less labor intensive than with other kelp species (Druehl & Clarkston 2016). This also means that surface monitoring of blade parameters (length, weight, proportion of blades with reproductive sorus present, etc.) encompasses a majority of individual biomass.

This study monitored *Nereocystis* canopy density, blade length, temperature, nitrate concentrations and abundances of a common SPS grazer (the northern kelp crab, *Pugettia producta*), in order to assess if temperature, nutrient availability and grazing pressure are associated with decreased blade lengths and bed densities. Oceanographic conditions in the Puget Sound display a strong geographic gradient with temperature decreasing, and nutrient concentrations and salinity increasing from south to north (Berry 2018). Given these known geographic gradients, we sought to answer two questions: Do environmental conditions, *Nereocystis* canopy density and plant condition differ between study sites? And, are differences in temperature, nutrient concentrations and grazing pressure correlated with differences in *Nereocystis* blade length? We hypothesized that environmental conditions, canopy density and blade length would differ along a geographic gradient with the most stressful conditions, shortest blade lengths and lowest plant densities occurring in the southernmost interior reaches of the SPS. We also hypothesized that high temperatures, low nitrate availability and dense aggregations of kelp crabs would be correlated to shorter blade lengths.

LITERATURE REVIEW

The ‘true’ kelps (order Phaeophyceae, class Laminariales) form large, biogenic, coastal habitats across the globe. These underwater forests serve as foundations for a stunning diversity of marine flora and fauna, engineering conditions that increase overall biodiversity. The largest species found in the northeast Pacific – *Macrocystis* and *Nereocystis* – grow tens of meters tall forming a dense floating canopy that slows water movement and reduces the amount of light reaching the forest floor (benthos).

Nereocystis is the only floating canopy forming kelp found in the Puget Sound and has been recently identified as critical habitat for the federally endangered Boccaccio (*Sebastes paucispinis*) and Yelloweye Rockfish (*Sebastes ruberrimus*) (National Marine Fisheries Service 2017). These forests are also crucial foundations for Puget Sound food webs that indirectly support healthy salmon and oca populations (Bertocci 2015). Kelps have also been identified as sentinel species, sensitive to temperature increases and water quality degradation (Steneck et al. 2002). Recent documented losses to Puget Sound bull kelp forests are cause for concern and identifying causes and consequences of these losses is a high priority to federal and local resource managers, and non-profits.

The Puget Sound is unique from an oceanographic perspective, composed of a number of fjordal basins and subbasins separated by narrow and shallow constriction points. Little is known about kelp in the context of this unique environment (Ebbesmeyer et al. 1988). However, the large body of available research on closely related species in similarly oceanographic climates provides important insight into the kelp response to biotic and abiotic stress (Schiel & Foster 2015; Steneck et al. 2002; Dayton 1985).

This literature review synthesizes available information on *Nereocystis*, Puget Sound kelp species and ecosystems as well as responses to stress from closely related kelp from other geographic and oceanographic contexts. It begins with a brief overview of the physical conditions common to the Puget Sound before providing a summary of the *Nereocystis* lifecycle and discussing the response of *Nereocystis* and other kelp species to common abiotic and biotic stressors. Finally, it concludes with a summary of available *Nereocystis* trend and distribution data and ongoing monitoring efforts in the Puget Sound.

Puget Sound: Place and Processes

Puget Sound is an estuary made up of four glacially scoured basins (fjords) connected by shallow, constricted passages: The Central Puget Sound, Whidbey Basin, South Puget Sound (SPS) and the Hood Canal (Ebbesmeyer et al. 1988; Fig. 1). The shallow waters of Admiralty Inlet separate the Strait of Juan de Fuca from the Puget Sound proper (waters inland of Admiralty Inlet) and Hood Canal. Admiralty Inlet also forms the junction between the Hood Canal, Central Puget Sound and Whidbey Basin. The Tacoma Narrows serves as the boundary between the Central and South Puget Sound.

The Central and South Puget Sound are considered well mixed systems, but the waters of the Hood Canal and Whidbey basin can become stratified due to less water mixing and large inputs of freshwater (Williams et al. 2001). A majority of water mixing occurs at constriction points due to turbulent, vertical mixing of the water column as water from deep basins is forced into shallow waters as it attempts to exit the basin of origin. As a result of this mixing, approximately 50 percent of water in a given basin is retained resulting in residence times of three to six months for dissolved materials (Ebbesmeyer et al. 1988).

Oceanographic conditions in the Central and South Puget Sound follow a distinct geographic gradient with waters becoming cooler and more nutrient rich as one travels north. These gradients are even detectable within basins – water temperatures were 2 to 3 °C cooler in the Tacoma Narrows, at the entrance to the South Puget Sound, than the waters of Budd Inlet near Olympia, WA (Berry et al. 2019).

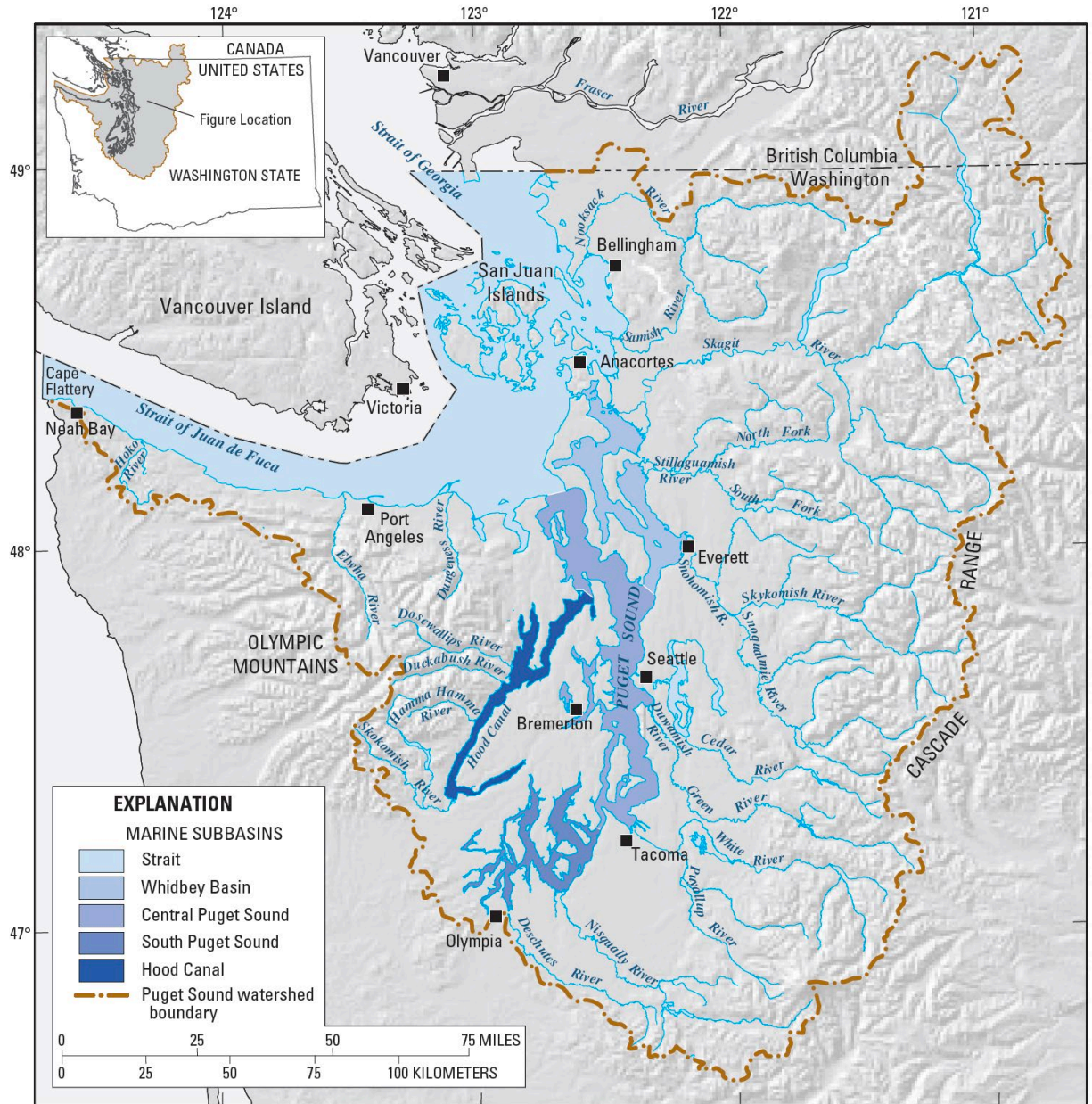


Figure 1: Map of the Puget Sound showing major basins (Gelfenbaum et al. 2006)

While environmental trends on a climatological scale explain long-term variation in kelp trends in the Strait of Juan de Fuca (Pfister et al. 2017), a review of the global kelp literature found that local environmental conditions explained local forest variation best (Krumhansl et al. 2016). This characteristic gradient of environmental conditions in the Puget Sound make it an ideal location for natural experiments to investigate the role of different environmental conditions on *Nereocystis* persistence and condition.

Nereocystis lifecycle

Like most kelp, *Nereocystis* exhibits a heteromorphic life history (Schiel & Foster 2006). This simply means that kelp alternate between macroscopic and microscopic lifestages. The *Nereocystis* lifecycle begins when an adult plant (diploid sporophyte) releases billions of zoospores (haploid spores) from special patches on the blades known as sori (singular: sorus) into the water. *Nereocystis* spore release follows a distinct diel pattern occurring during the few hours before and after sunrise and begins with sorus abscission resulting from cell necrosis around the sorus perimeter (Amsler & Neushul 1990). Kelp generally require hard substrates and zoospores will attach readily to both consolidated bedrock and unconsolidated gravel, in some cases kelp spores even attach to other macroalgae (Dayton 1985). Once attached, *Nereocystis* spores germinate into male and female gametophytes (in as quickly as one week, although the longevity in nature of the gametophyte plants is not known) that reproduce sexually to produce microscopic, germling sporophytes (after approximately three weeks). Following this initial recruitment juvenile sporophytes grow rapidly throughout the entirety of the growing season (Maxell & Miller 1996).

Generally, kelp spores fall close to the parent plant, although spores can be carried further (up to several kilometers) depending on local current conditions and the depth at which spores are released (Gaylord et al. 2002). Considering that *Nereocystis* sorus production occurs at the water surface, it is likely that spore dispersal distances for *Nereocystis* may be significantly larger than for other species. Additionally, adult plants broken free from the benthos can form floating rafts capable of successfully producing viable spores (Rothäusler et al. 2009).

For all *Nereocystis* and other annual species as well as perennial species that recruit according to predictable seasonal patterns, there is evidence that microscopic forms remain dormant or overwinter until conditions are favorable for reproduction and growth (Carney & Edwards 2006). Generally, evidence points to gametophytes being the most common lifestages capable of overwintering although there is some evidence that spores and germling sporophytes may as well. This is especially the case for *Nereocystis* and other annuals as adult plants are often totally absent for a portion of the year. This study focuses on the response of adult *Nereocystis* sporophytes to common stressors and from this point forward *Nereocystis* will refer to the adult sporophyte stage unless otherwise specified.

Nereocystis produces sori on apical blades that float near the surface, whereas sori of *Macrocystis* sporophytes are found on specialized blades called sporophylls near the benthos (Druehl & Clarkston 2016). This means that all *Nereocystis* sorus production occurs in the first few meters of the water column. As a result, surface conditions likely exert more influence over the reproductive potential of *Nereocystis* than other kelp species.

Nereocystis is often described as a ruderal and early successional species and it quickly recruiting to new substrate following disturbances (Suskiewicz 2010; Dayton et

al. 1992). This has allowed for insight into the response of *Nereocystis* to a variety of disturbances as full successional cycles can be manipulated and observed over the course of a few years—a stark contrast to terrestrial forest research which requires observations over decades (Duggins 1980). However, the alternation of heteromorphic generations characteristic of the *Nereocystis* life-cycle complicates attempts to understand the full impacts of environmental and biological stress as environmental requirements may differ between microscopic and macroscopic lifestages (Steneck et al. 2002).

Nereocystis forests, like all kelp forests, engineer resilient foundations for complex, diverse and productive nearshore ecosystems (Bertness et al. 2014; Hurd et al. 2014). Due to its annual nature, many *Nereocystis* canopies are often completely absent from late fall to early spring with some, in the more oceanic waters of the Strait of Juan de Fuca persisting for one to two years (Maxell & Miller 1996; personal observations). Variation in canopy extent and density is influenced by a combination of environmental and biological factors. Broadly, interactions between environmental conditions, physical forces and biotic interactions work to constrain or promote kelp forest structure (Pfister et al. 2017; Steneck et al. 2002). These interactions are often complex and further complicated by the synergistic effects of multiple stressors (Crain et al. 2008).

Kelp forests have also played a large role in providing evidence for biotic controls on ecosystems, specifically top-down trophic effects from keystone predators. Most well documented are the interactions between sea otters (*Enhydra lutis*) and urchins (*Strongylocentrotus spp.*) (see Steneck et al. 2002). However, this classic dynamic likely does not pertain to Puget Sound kelp forest as urchin populations aren't as robust as in the Strait of Juan de Fuca and along the outer coast.

Abiotic controls: Environmental conditions and physical forces

Photosynthetic performance

Nereocystis requires light in order to carry out photosynthesis, grow and reproduce (Hurd et al. 2014; Dayton 1985), and lack of sufficient light is one stressor that can negatively affect *Nereocystis*. Kelp occupy the photic zone, the area of the nearshore environment where light penetrates to the benthos and photosynthesis occurs at variable rates over a range of irradiances (Hurd et al. 2014).

At compensation irradiances, photosynthetic rates match cellular respiration (Wiencke et al. 2006). The compensation level of irradiance for kelp is generally 2 to 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (0.01 to 0.05 percent of surface light on a sunny day) (Hurd et al. 2014). Even though growth may still occur below compensation irradiances, the ability of any kelp lifestages to deal with additional stressors is compromised. In addition, sporophyte sorus production, spore germination, gametophyte reproduction and germling saprophyte growth may be delayed or impeded at such low irradiances (Carney & Edwards 2006; Vadas 1972). For example, germling *Macrocystis* sporophytes delay growth between 2 to 3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Carney & Edwards 2006). Similarly, Vadas (1972) found that only 1 percent of *Nereocystis* gametophytes attained fertility when exposed for three weeks to irradiances of approximately 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Photosynthetic rates increase with increasing irradiance until maximum photosynthetic rates are attained at saturation irradiances. At this point, any increase in irradiance returns little to no increases in photosynthetic rates. Saturation irradiances for kelp fall between 150 to 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Hurd et al. 2014). In laboratory cultures,

Nereocystis gametophytes and germling sporophyte growth rates peaked between approximately 15 to 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, similar to the critical levels needed to induce germling sporophyte growth in *Macrocystis* (20 to 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Carney & Edwards 2006; Vadas 1972).

While light is essential for photosynthesis, photo-inhibition can occur when high irradiances and UV exposure lead to cellular damage and tissue death. Photo-inhibition for kelp in general occurs between 850 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with microscopic stages being especially susceptible to UV damage (Swanson & Druehl 2000). However, photo-tolerance, like many traits, is species specific and no data exists for any *Nereocystis* lifestage.

Temperature

On a global scale, kelp species are distributed along latitudinal temperature gradients (Hurd et al. 2014; Bartsch et al. 2008; Lüning & Freshwater 1988). Individual species have unique optimal temperature ranges that can also differ between alternative lifestages (Hurd et al. 2014; Harley et al. 2012; Dayton 1985). Temperature optimums can be difficult to fully describe as individual species can adapt and acclimate to temperature stress to different degrees (Lind & Konar 2017; Dayton 1985). However, trends show similar optimal ranges for species with similar distributions.

Other cold-temperate kelp species related to *Nereocystis* in the genus *Laminaria* can survive and reproduce from 0 to 18 °C but grow optimally in the range of 5 to 15 °C (Bartsch et al. 2008). Similarly, while *Nereocystis* sporophytes can survive at a range of -1.5 °C to 18 °C (Lüning & Freshwater 1988), Maxell & Miller (1996) found that Puget

Sound *Nereocystis* stipe and blade growth rates peaked in concert with summer time high temperatures of 13.5 °C.

Power plants in California with outflow pipes terminating in coastal waters are required to comply with rigorous environmental monitoring practices. As a part of regulatory compliance, laboratory experiments were undertaken to understand *Nereocystis* response to temperature (Springer et al. 2010). Temperatures exceeding 18 °C resulted in total mortality of adult sporophytes (Tera Corp. 1982). However, even exposure to slightly elevated temperatures of 15.9 °C resulted in 25 percent mortality of adult sporophytes over approximately a month (Tera Corp. 1982). The authors attributed this mortality to the inability of *Nereocystis* to repair physical damage from handling during flume experiments. These laboratory results were corroborated by Schiel et al. (2004) after an analysis of community changes over ten years in response to water temperature increase of 3.5 °C in a kelp forest adjacent to a powerplant outflow.

Increases in water temperature coincided with a 90 percent loss of the midcanopy species *Pterygophora californica* and a 97 percent loss of *Nereocystis* in California (Schiel et al. 2004). However, loss of adult sporophytes is not antithetical to kelp persistence. As mentioned, kelp is resilient to disturbance and microscopic lifestages show the ability to remain dormant until conditions favor growth (Edwards 2000).

Similarly, Wernberg et al. (2010) explored the relationship between disturbance, temperature and canopy recovery along a naturally occurring latitudinal temperature gradient along the west Australian coast. Temperatures ranged from between 2 to 4 °C between sites allowing the researchers to explore the effects of predicted sea surface temperature rise on the Australian canopy species *Ecklonia radiata*. While temperature did

not affect canopy cover or total biomass of beds, it did significantly impact recruitment following disturbance. This suggests that temperature tolerances of microscopic lifestages are significantly lower than those of adult plants and that even small increases in oceanic temperatures over the long-term could reduce overall kelp resiliency.

Outside of optimal temperature ranges, photosynthetic performance can be augmented by increasing pigment content, reaction centers, and protein complexes allowing for higher maximum photosynthetic rates and yield (Bartsch et al. 2008). However, maintaining positive growth in the face of temperature stress may make adult sporophytes more susceptible to additional disturbances (Wernberg et al. 2006). This is in part due to the fact that photosynthetic rates can only be increased so much before respiration and biomass loss outpaces photosynthetic yields (Hurd et al. 2014).

For example, Krumhansl et al. (2014) used field observations of kelp detrital production across seasonal temperature variations to model detrital production response to predicted climate change. They found that as temperatures warm, kelp will provide increasing levels of detritus until the point at which tissue degradation outpaces growth. Similarly, Rothäusler et al. (2009) monitored experimental rafts of *Macrocystis* along a latitudinal temperature gradient on the Chilean coast. Rafts kept at temperatures of 12 to 19 °C grew and reproduced successfully while those kept at temperatures > 20 °C began to degrade and did not produce reproductive sori.

Nutrients

Seasonal and geographic variations in nutrient availability have the potential to influence the health and productivity of *Nereocystis* (Schiel & Foster 2006; Dayton 1985).

Like other marine aquatic vegetation and kelp species, *Nereocystis* is nitrogen limited (Hurd et al. 2014; Dayton 1985). In laboratory investigations of nutrient uptake rates, Ahn et al. (1998) found that *Nereocystis* showed preference for nitrate over ammonia as nitrate uptake rates increased linearly with nitrate additions while ammonium uptake rates peaked at around 10 μM of ammonium. This preference for nitrate holds true for other closely related kelp species such as *Macrocystis* and kelp species in the genus *Lamiaria Sensu Lato* (Schiel & Foster 2015; Bartsch et al. 2008).

The majority of nutrient transport into the Puget Sound occurs via deep water influent of oceanic waters through the Strait of Juan de Fuca (Khangaonkar et al. 2018). Transport of nutrients from deeper, oceanic waters to surface waters is a slow process but the Puget Sound is, overall, a well-mixed system, thanks to regular vertical mixing occurring at shallow constriction points between basins (Ebbesmeyer et al. 1988). Generally, it is accepted that nitrate availability is inversely related to temperature (Dayton 1985). Along the outer coast and Strait of Juan de Fuca, this is because nutrient upwelling regimes are often interrupted during periods of high temperatures. Whether this relationship holds true for all reaches and embayments of the Puget Sound is unknown and may only be an issue for shallower and less mixed reaches.

Biotic controls in the SPS

Anecdotal evidence from local residents, tribal members and environmental professionals document increases in the numbers of the kelp crab *Pugettia producta* in the central and SPS. These accounts often go hand in hand with observations of canopy loss in the same regions. Recent research by Dobkowski (2017) confirms *P. producta* grazing

preference for *Nereocystis* over other locally abundant kelp and specifically a preference for juvenile sporophytes over adults, but does not document *P. producta* impacts to *Nereocystis* recruitment or growth. In California, multiple year classes of kelp crabs (as determined by comparisons of carapace size) are found coexisting in floating canopies (Hines 1982). Similar observations made at SPS *Nereocystis* forests documented little variation in carapace size with nearly all crabs starting the season as juveniles and maturing over the course of the summer (Berry et al. 2019). This may suggest that kelp crabs recruit annually with the appearance of *Nereocystis*. Little is known regarding adult kelp crab habitat preference in the Puget Sound or what effect adult populations may have on early kelp recruitment.

There is evidence in the literature that environmental conditions can interact with relative grazer abundances to influence the mortality and health of kelp (Rothäusler et al. 2009; Duggins et al. 2001). Duggins et al. (2001) investigated the role of current flow on grazer abundance of the molluscan gastropod *Lacuna vincta* and *Nereocystis* mortality in the San Juan Islands of Washington state and found that even minor damage to *Nereocystis* stipes significantly reduced their breaking strength when exposed to high current velocities. Pfister & Betcher (2017) observed similar patterns of mortality associated with wave action and grazer damage to stipes of the upper subtidal species *Pleurophycus gardneri* along the coast of Tatoosh Island off the coast of Washington. However, interactions between grazing and other kelp stressors is not limited to current velocity alone.

As discussed previously, temperature stress can reduce the resiliency of kelp to additional stressors by negatively impacting physiological ability to repair damaged tissue (Krumhansl, K. A. et al. 2014; Harley et al. 2012). To investigate the interactions of grazer

stress and temperature Rothäusler et al. (2009) observed the impact of temperature and grazing on biomass of floating *Macrocystis* rafts along the Chilean coast. At intermediate temperatures of 16 to 19 °C, rafts without grazers maintained or increased biomass while rafts exposed to grazers steadily lost biomass suggesting that temperature stress reduces overall resiliency of adult sporophytes.

Washington State kelp

Our current understanding of the state of Puget Sound kelp resources is largely restricted to floating *Nereocystis* canopies as they can be easily surveyed from boats, aircraft and satellites. Little information exists regarding distributions of the remaining 20+ Puget Sound kelp species in Puget Sound (Mumford 2007). Traditional ecological knowledge from tribes and local residents, citizen-science surveys, and analysis of historical data points to significant declines in the extent of *Nereocystis* forests throughout the Puget Sound (Palmer-McGee 2019; Berry 2018; Berry et al. 2005).

Local residents encountered at docks and boat launches throughout the state often offer personal accounts of losses to *Nereocystis* forests (personal communication). Whether individuals encountered are recreational boaters, avid beachcombers, management agency officials or tribal members, most recall a greater abundance and area of *Nereocystis* canopies in the 1970's and 1980's than today. When asked about possible explanations, responses vary from increases in kelp crab abundance and losses to important fisheries species to effects of shoreline development and climate change.

Comparisons of long-term aerial photography of the north Olympic Peninsula to kelp surveys from 1911 and 1912 document decreases in kelp canopy extent and area

around Dungeness Spit, Protection Island, and Port Townsend (Pfister et al. 2017). Similar comparisons between 2016 aerial photography and 2006 remote sensing data document a 36 percent decrease in *Nereocystis* canopy area in the San Juan Islands (Palmer-McGee 2019).

In the South Puget Sound (SPS), recent and comprehensive analysis of historical kelp surveys, navigation charts, and incidental kelp observations from habitat and other target species surveys show clear changes to *Nereocystis* canopy extent (Berry 2018). Linear extent of kelp canopies and area have declined approximately 68 percent in the SPS (Berry 2018). The Washington Department of Natural Resources (WA-DNR) is currently working on analyzing historical data in order to parse out *Nereocystis* canopy trends for the Central Puget Sound but the Puget Sound Restoration Fund (PSRF) has already documented a total loss of *Nereocystis* canopies around Bainbridge Island and from the Kitsap Peninsula around Jefferson Head (PSRF, personal communication).

Comprehensive inventories of all kelp species found in Puget Sound are nonexistent. WA-DNR has taken first steps toward understanding the full extent and area of Puget Sound *Nereocystis* canopies by identifying and mapping all beds in the SPS during the summer of 2017. Similar inventories are slated for the Central Puget Sound during summer of 2019 (Berry, personal communication). In addition, WA-DNR is undertaking the first assessment of non-floating kelp extent using towed underwater video taken in the summer of 2018 of the entire shallow, subtidal King County coast. WA-DNR has also monitored declines in forest area, depth extent, and kelp health at the Tucksel Point *Nereocystis* forest at Squaxin Island from 2013 to present (Berry 2017).

In addition to WA-DNR's efforts, the Northwest Straits Commission (NWSC) and seven county Marine Resource Committees (MRCs) have implemented protocols for citizen-science kayak mapping of *Nereocystis* canopies (Bishop, n.d.). Surveys conducted in 2014 of kelp area in the Strait of Juan de Fuca, Smith and Minor Islands, Cypress Island, and Cherry Point all documented decreases in canopy area. Kelp canopy area recovered in 2015 and 2016 in well-mixed areas, but not at the more sheltered site at Cherry Point. In addition, the Snohomish County MRC has documented losses of several beds and declines in remaining beds between Edmonds and Mukilteo (personal communication).

Current assessments of the extent and abundance of kelp canopies are, so far, rarely paired with monitoring of key abiotic and biotic conditions within associated kelp forests. Local conditions play a significant role in the large interannual variability of kelp forest extent and abundance, and Puget Sound encompasses a number of sub-basins and reaches with distinct environmental conditions (Krumhansl et al. 2016; Ebbesmeyer et al. 1988). Thus, causes for declines in one region may not be applicable to Puget Sound as a whole.

This study is an early attempt to document differences in forest condition in response to key stressors known to exert significant influences on the persistence and health of kelp forests elsewhere. Surveys adapted existing WA-DNR methods for monitoring plant condition and density at sites in the Puget Sound and paired these observations with assessments of environmental conditions and grazer abundance. Pairing observations of forest condition with records of environmental and community interactions may be used to identify priority stressors at local sites through the Puget Sound and develop mitigation and conservation strategies to protect remaining *Nereocystis* canopies and understory assemblages.

METHODS

Site Selection

Monitoring was conducted between 2017 and 2018 in the South Puget Sound (SPS), Washington. The SPS is the shallowest and most inland portion of the Puget Sound and is separated from the Central Puget Sound by the Tacoma Narrows (Fig. 1). As is the case in much of the Puget Sound, all sites were characterized by high current velocities (often exceeding 100 cm sec^{-1} during maximum tidal exchanges) tied to large tidal shifts (often $>3\text{m}$).

A total of four sites were selected for monthly temperature and nitrate monitoring (Fig. 2). The sites were distributed south to north from Budd Inlet, Olympia, Washington to Salmon Beach, Tacoma, Washington in order to encompass a majority of SPS *Nereocystis* forests.

From south to north the sites were: Tucksel Point located at the southern tip of Squaxin Island (lat. $47^{\circ}10'0.49''\text{N}$, long. $122^{\circ}53'34.54''\text{W}$); Devils Head on the southern Kitsap Peninsula (lat. $47^{\circ}9'58.42''\text{N}$, long. $122^{\circ}45'37.11''\text{W}$); Day Island south of Titlow Beach in the Tacoma Narrows (lat. $47^{\circ}14'21.78''\text{N}$, long. $122^{\circ}33'52.00''\text{W}$) and Salmon Beach, north of the Tacoma Narrows bridge (lat. $47^{\circ}17'34.58''\text{N}$, long. $122^{\circ}31'48.00''\text{W}$).

Three sites (Squaxin Island, Devil's Head and Day Island) were initially selected for intensive monitoring *Nereocystis* individuals and bed density during the usual growing season (May to September). Intensive monitoring protocols were conducted at Salmon Beach in July and September 2018 after the Devil's Head canopy failed to appear in June. At all intensive monitoring sites, two along-shore transects were used to establish 15 sets of paired plots within previously surveyed 2017 bed perimeters. Paired plots consisted of

a near-shore (shallow) and off-shore (deep) plot to attempt to encompass total depth strata of each bed while covering as much of previous year's bed area as possible (Fig. 3). Three monitoring points were established along the offshore side of each bed at a depth of -7 MLLW and revisited monthly from May 2017 to September 2018. Casting locations were independent from the 15 paired plots established for intensive monitoring. Casting stations were located offshore of the north, central and southern sections of each *Nereocystis* forest (Fig. 3).

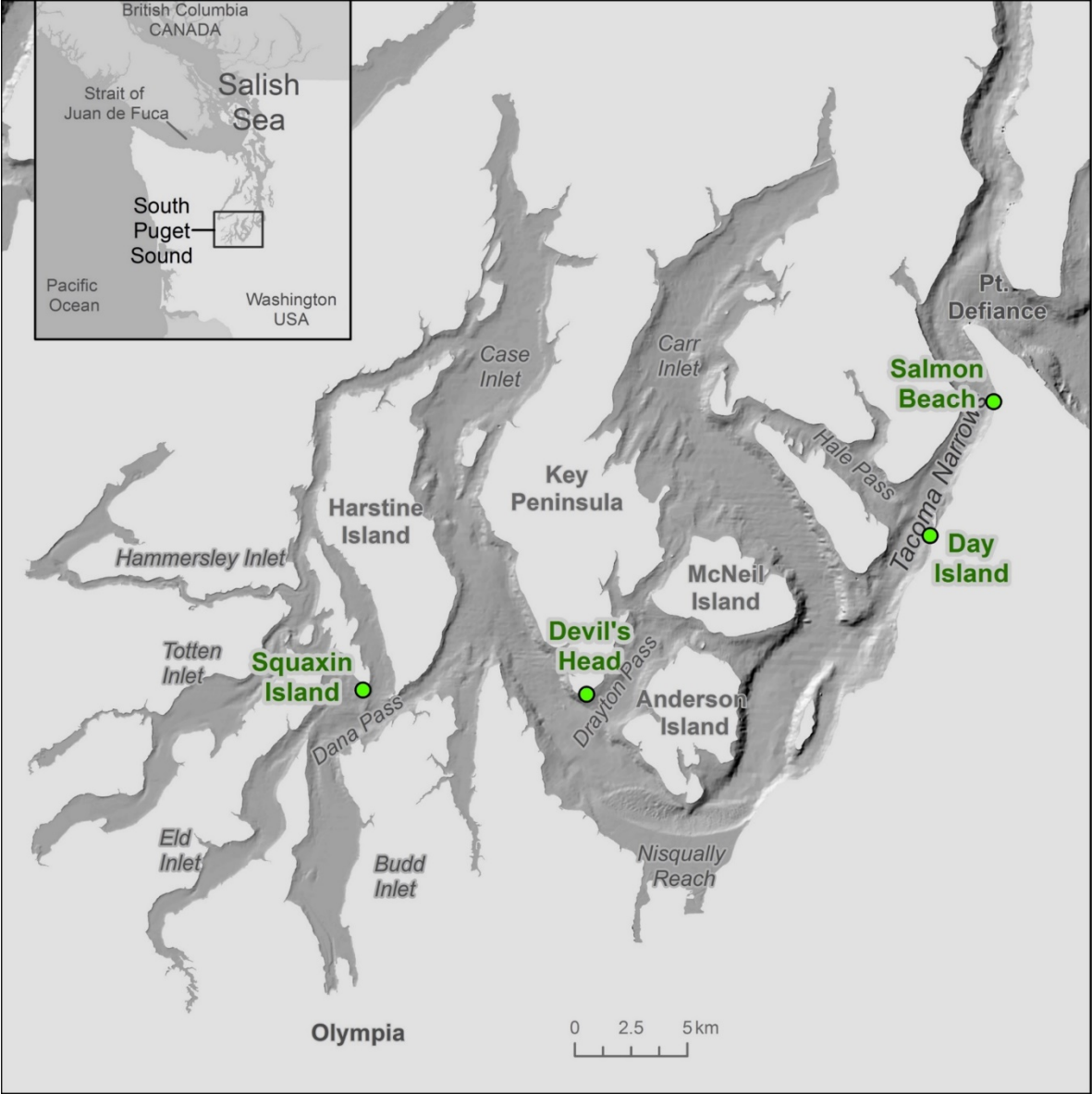


Figure 2: South Puget Sound and study sites.

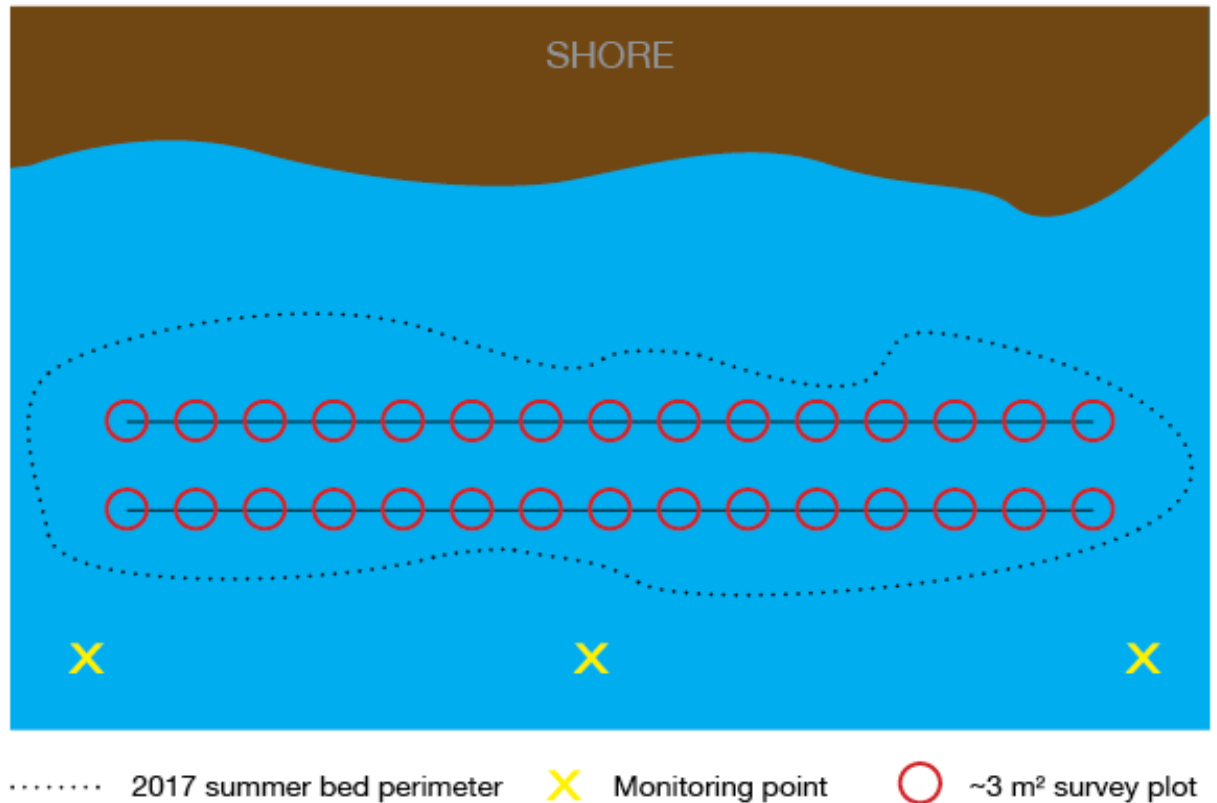


Figure 3: Site monitoring design.

Mean Water Column Temperature

Monitoring of water temperature occurred monthly in partnership with the Washington Department of Natural Resources (WA-DNR) Nearshore Habitat Program. All sites were surveyed on the same day, within two hours before and after solar noon.

Temperature and salinity were measured using a weighted *SonTek Castaway*®-CTD instantaneous data sonde. The sonde relies on flow-through sensors to log instantaneous temperature and salinity data while free-falling through the water column at a rate of approximately 1 m s⁻¹. After each cast, data was quality checked in the field using the sonde's real-time data display. In the event of irregularities in cast depth profiles, the

sonde was recast to ensure data quality. Integrated water column temperatures were averaged across all three casting sites.

Nitrate availability

Monthly field filtered water samples were collected from the central monitoring point at each site for analysis of nitrate concentrations. An acid washed 60 mL syringe with an attached 0.45 μm cellulose acetate filter was filled with water directly from a Van Dorn sampler. A small amount of water was filtered through the syringe to rinse the syringe and syringe filter before rinsing an acid washed 60 mL high density polyethylene (HDPE) bottle with filtrate. The bottle was then filled with filtrate before being placed immediately in a cooler on ice and transported to the Evergreen State College laboratory where they were frozen (-10°C) for later transport to the University of Washington's Marine Chemistry Lab for total dissolved nutrient analysis using continuous flow automated colorimetric analysis (Knap et al. 1996).

In March only one sample was taken from each site from a depth of -4 m MLLW due to the relatively low water temperatures observed during sampling. From May to September two samples were taken from each site at depths of -0.25 m MLLW and -4 m MLLW in order to detect if nitrate concentrations on the surface differed from water column in order to assess possible effects of seasonal thermal stratification.

Bed-wide and *Nereocystis* blade length monitoring

Monitoring of *Nereocystis* and kelp crab (*Pugettia producta*) densities and individual *Nereocystis* blade length began in May when adult plants were first visible at

the water surface during daytime low tides (≤ 0 m MLLW). Monitoring continued monthly during spring tide windows with water levels ≤ 0 m MLLW. All bed and individual monitoring occurred one hour before and after predicted low tide during the slack tide window to minimize interference from current velocities during the survey process. The 15 paired plots established within previously mapped 2017 bed perimeters were used to monitor *Nereocystis* and kelp crab (*Pugettia producta*) densities, and the selection of one *Nereocystis* individual selected per point for morphometric measurements.

At each point, demarcated kayak paddles were used to delineate a 3.79 m² circular quadrat. The paddle was positioned at stomach level with the middle of the paddle serving as the center of the circular quadrat. Plants directly under the observer could not be seen but those under the stern and prow of the kayak could be easily counted. Within each quadrat, all individual *Nereocystis* bulbs were enumerated, as well as any kelp crabs present in the plot (regardless of associated substrate).

The *Nereocystis* individual closest to the off-shore side of the kayak was selected for individual blade length analysis at each point. At points with density counts of zero, the closest plant within 4 m of the point was selected. If no plant fell within this extended radius, no individual was measured for blade length. Blade length was recorded only for the longest blade present on an individual. If the longest blade was more than 0.5 m longer than the second longest blade, the second longest blade was measured.

Analysis

All data were analyzed using the R statistical programming language (Version 3.5.1, R Core Team 2018). Plant and crab densities per m² were calculated by dividing raw plant

count data by the area of the sampling quadrat. Plant density and crab density were positively skewed and contained a large number of zeros. Temperature and nitrate concentration data were non-normally distributed with no obvious skew to the data. One-way ANOVAs were used to test for differences in plant density, crab density, water temperature and nitrate concentrations despite deviations from normality due to the robustness of the test in the face of non-normally distributed data (Schmider, E. et al. 2010).

Plotted residuals from the one-way ANOVAs investigating differences in mean plant density between sites and between sites through time were skewed right and an investigation of the models revealed significant heteroscedasticity in the data as determined using a Bruesch-Pagan test from the `lmtest` package (Zeileis, A. & T. Hothorn 2002). Due to the inclusion of zeros in the data set, a Tukey's power transformation from the `rcompanion` package was selected over a logistic or Box-Cox transformation (Mangiafico, S. 2019).

One-way ANOVAs using the transformed density data rectified issues of non-normally distributed residuals and heteroscedasticity for mean plant density through time but not for comparisons of mean plant density between sites. As a result, a Welch's ANOVA with Games-Howell post hoc pairwise comparisons (`biostat` package; Gegzna, V. 2018) were used to determine differences in plant density between sites while a one-way ANOVA with Tukey's power transformed plant density data was used to determine differences between sites through time.

Similarly, one-way ANOVAs using both crab density and Tukey's power transformed crab density data suffered from non-normally distributed residuals and heteroscedasticity while transformed temperature and nitrate concentration data suffered

from heteroscedasticity only. Thus, a Welch's ANOVA with Games-Howell post hoc test was used to determine differences in average crab density and nitrate concentration between sites as well as differences in water temperature between sites through the season. Due to the low sample sizes for nutrient concentrations and high month-to-month variability in crab densities, no analyses were performed to investigating changes in nutrient concentration or crab densities from month to month.

Differences in mean blade length between sites and across time was investigated using a one-way ANOVA with a post hoc Tukey's test used to assess pairwise differences.

A linear regression with a quadratic polynomial treatment of temperature was used to assess the effect of temperature, crab densities and nitrate availability on blade length across all sites. However, considering the significant differences in explanatory and response variables between sites, individual, site specific models were developed in addition.

RESULTS

Survey Timing

Surveys of bed density and plant morphometrics began on 20 May 2018 at Squaxin Island and 31 May 2018 at Day Island after the first mature sporophytes recruited to the surface. A surface canopy was absent at Devil's Head on 18 May 2018 and 26 June 2018 and, as a result, Devil's Head was excluded from bed density and individual *Nereocystis* blade condition analyses.

Despite the decision to discontinue canopy surveys at Devil's Head, we felt that it was important to conduct in-water observations in order to ascertain if the absence of a

surface canopy was due to an absence of *Nereocystis* sporophytes, or if a sub-surface canopy existed. A subsurface canopy was encountered during a snorkel survey of the southern half of the known 2017 bed area but only five plants were observed. With no surface canopy at Devil's Head, surveys began at Salmon Beach on 26 July 2018 in order to ensure three sites for statistical comparisons of bed density and individual *Nereocystis* blade condition.

No surface canopy at Devil's Head was observed during temperature and water nutrient sampling on 18 July 2018 or while passing by Devil's Head on the way to Salmon Beach on 26 July 2018. A SCUBA survey was undertaken on 13 August 2018 at Devil's Head to determine if the subsurface canopy had persisted through July, but a visual encounter survey conducted along a transect bisecting the 2017 bed perimeter longitudinally found no *Nereocystis*.

Salmon Beach is located in the Tacoma Narrows, the shallow and narrow constriction separating the SPS from the Central Puget Sound (Ebbesmeyer, C. C. et al. 1988). This area is characterized by high tidal currents and is far from public boat launches making kayak surveys contingent on motorized boat support. No Salmon Beach survey was conducted in August due to a lack of boat support.

No blades were found on any of the *Nereocystis* individuals surveyed on 24 August 2018 at Squaxin Island. As a result, surveys were discontinued, and no survey was conducted in September.

Site differences

Plant Density

Plant densities differed significantly between sites as determined by Welch's ANOVA ($F_{2,127.25}=31.9, p < 0.001$). Densities at Squaxin Island were significantly lower ($M = 0.57 \text{ m}^{-2}, SE = 0.09$) than at either Day Island ($M = 2.26 \text{ m}^{-2}, SE = 0.24, p < 0.001$) or Salmon Beach ($M = 1.96 \text{ m}^{-2}, SE = 0.26, p < 0.001$) (Table 1, Fig. 4). Mean aggregate plant densities were similar at Day Island and Salmon Beach.

Table 1. Plant density per square meter as a function of site: Sample size, mean, standard deviation, standard error, median, first quartile, third quartile and interquartile range.

Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	<i>MED</i>	<i>Q1</i>	<i>Q3</i>	<i>IQR</i>
Squaxin Island	64	0.57	0.73	0.09	0.26	0.00	0.79	0.79
Day Island	108	2.26	2.55	0.24	1.06	0.53	3.43	2.90
Salmon Beach	50	1.96	1.81	0.26	1.32	0.79	2.57	1.78

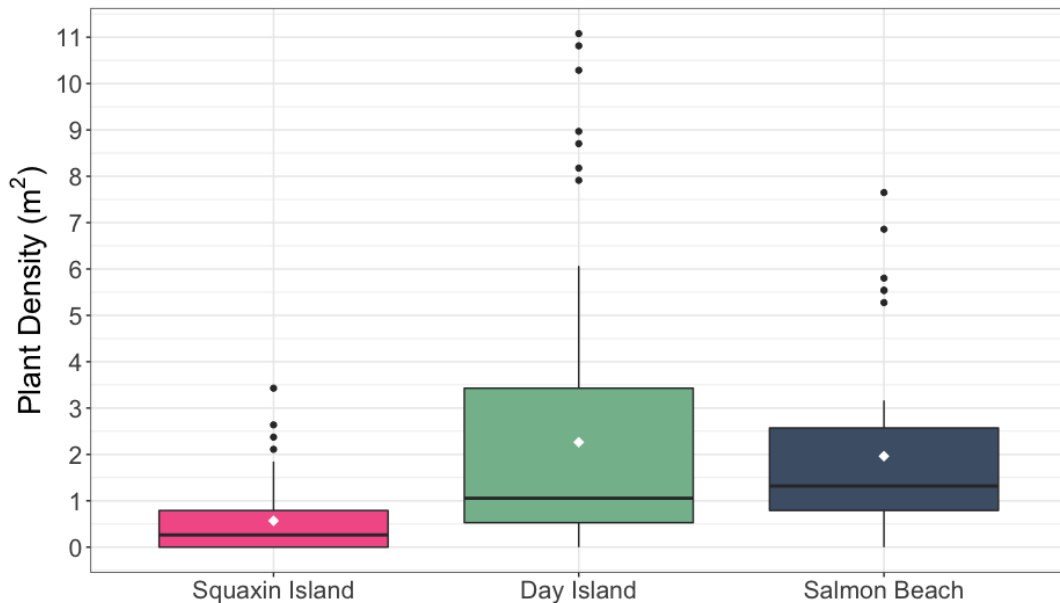


Figure 4. Plant densities at each site (black line = median, white diamond = mean).

Decreases in plant density were also observed between months over the course of the growing season (one-way ANOVA, $F_{10,211} = 9.43$, $p < 0.001$), with plant densities declining at all sites each month (Table 8, Fig. 5). A Tukey's HSD post hoc test was run to determine differences between sites during the same month and within sites from month to month.

Density surveys in May at Day Island captured initial high density ($M = 5.8 \text{ m}^{-2}$, $SE = 1.16$) recruitment to the canopy characteristic of *Nereocystis* (Dobkowski et al. 2019) followed by a significant decrease in June ($M = 2.35 \text{ m}^{-2}$, $SE = 0.5$, $p < 0.001$). Densities at Salmon Beach were slightly higher than those at Day Island for months where data is available, and it is likely that the higher seasonal average plant density observed at Day Island is the result of the initial high densities observed in May. Squaxin Island consistently had significantly lower densities compared to Day Island and Salmon Beach for all months with available data ($p \leq 0.01$ for all pairwise comparisons). Finally, Squaxin Island bed density in August ($M = 0.3 \text{ m}^{-2}$, $SD = 0.1$) was significantly lower than September bed density at Salmon Beach ($M = 1.54 \text{ m}^{-2}$, $SE = 0.27$, $p = 0.003$).

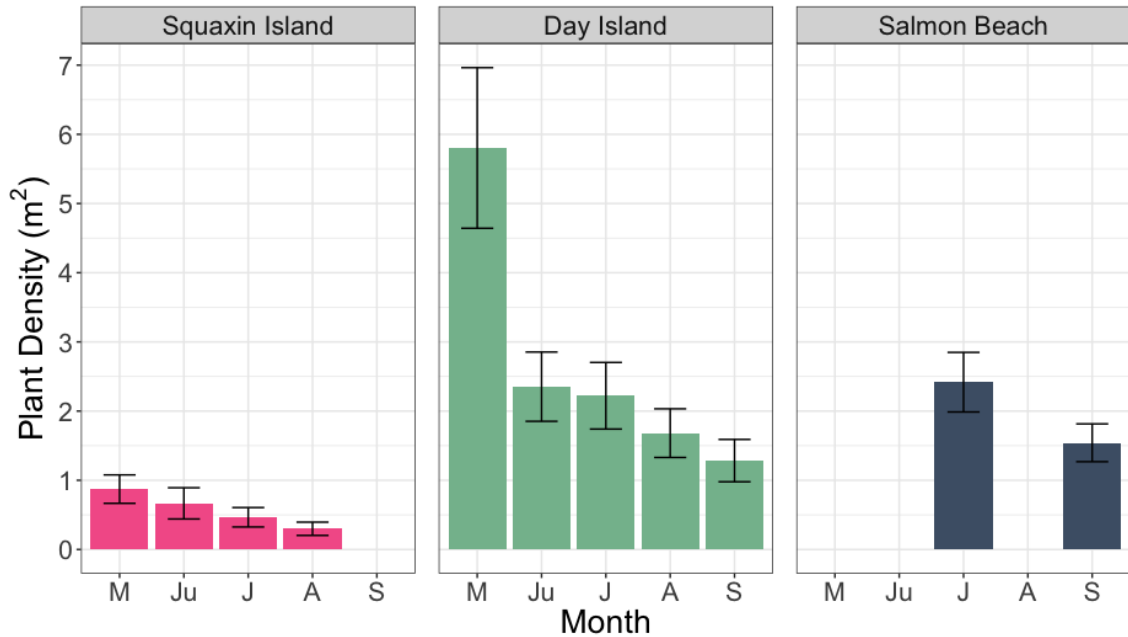


Figure 5. Monthly plant densities at each site (mean \pm SE).

Blade Length

Overall, mean growing season blade lengths differed significantly between sites (one-way ANOVA, $F_{2, 171} = 32.07$, $p < 0.01$) as well as throughout the growing season (one-way ANOVA, $F_{8, 165} = 18.06$, $p < 0.01$). Average blade length at Salmon Beach ($M = 3.34$ m, $SE = 0.16$) was significantly longer than both Squaxin Island ($M = 2.37$ m, $SE = 0.15$, $p < 0.001$) and Day Island ($M = 2.06$ m, $SE = 0.08$, $p < 0.001$) as determined by a Tukey's HSD post hoc test (Table 2, Fig. 6). Average blade length at Squaxin Island was the shortest of all sites but not significantly shorter than at Day Island.

Table 2. Blade length (m) as a function of site: Sample size, mean, standard deviation, standard error and 95% confidence interval for

Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	95% <i>CI</i>
Squaxin Island	38	2.37	0.91	0.15	2.08 – 2.66
Day Island	89	2.06	0.76	0.08	1.90 – 2.22
Salmon Beach	49	3.34	1.10	0.16	3.03 – 3.64

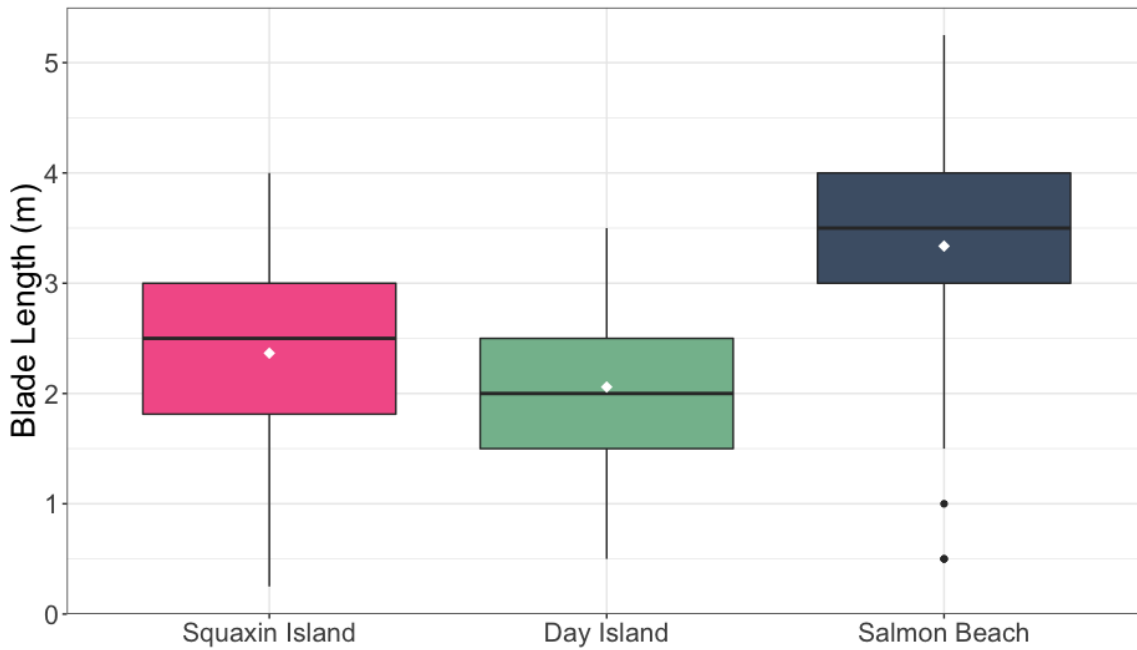


Figure 6: Blade length at each site (black line = median, white diamond = mean).

Monthly average blade length differed significantly between sites during the 2018 growing season (one-way ANOVA, $F_{9,166} = 16.18, p < 0.01$). A Tukey’s HSD post hoc test was used to determine differences between sites on the same month and within sites between months. Blades lengths at both Squaxin and Day Island increased at the beginning of the season, hitting peak length in June and July respectively before beginning significant declines (Table 9, Fig. 7). Early season blade lengths recorded in May and June at both sites did not differ significantly from one another. However, by July, Squaxin Island blade

length ($M = 1.69$ m, $SE = 0.18$) was significantly shorter than at Day Island ($M = 2.68$, $SE = 0.12$, $p = 0.007$) and Salmon Beach ($M = 3.51$, $SE = 0.17$, $p < 0.001$).

Observations of longest blade lengths at Squaxin Island occurred in June ($M = 2.99$ m, $SE = 0.15$) with significant declines occurring in July ($M = 1.69$ m, $SE = 0.13$, $p < 0.001$) followed by a total loss of blades on all plants surveyed in August. At Day Island this general trend was delayed by one month, with peak blade lengths observed in July ($M = 2.68$ m, $SE = 0.12$) followed by significant declines in August ($M = 1.83$ m, $SE = 0.16$, $p = 0.04$) and further declines September ($M = 1.34$ m, $SE = 0.13$, $p = 0.63$).

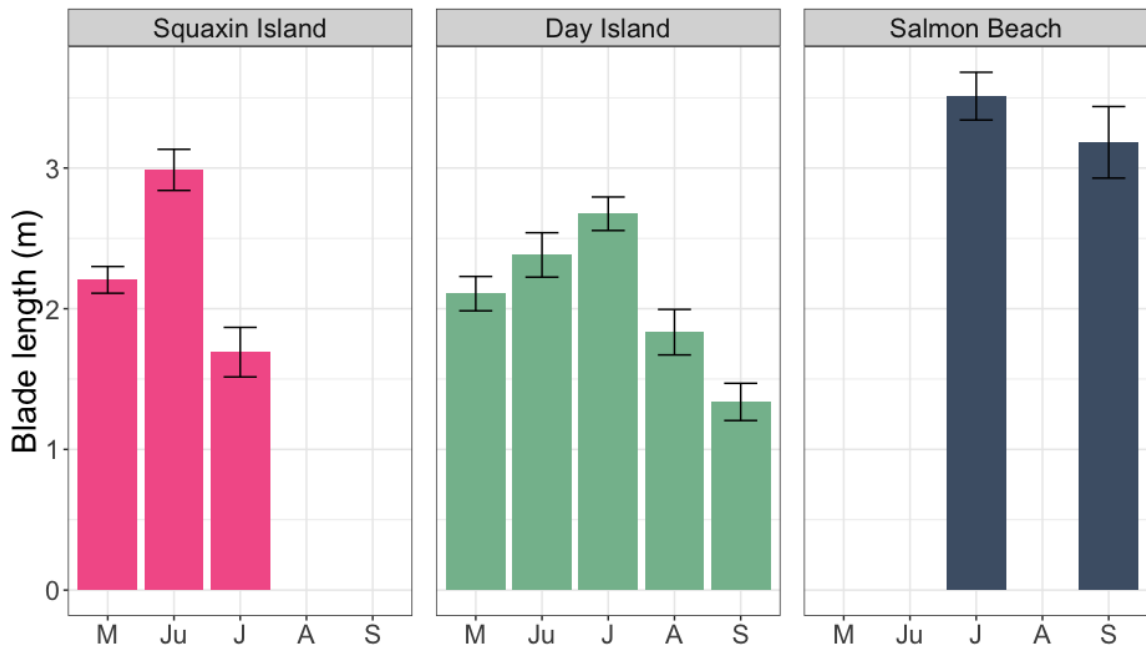


Figure 7: Average monthly blade lengths of *Nereocystis* individuals at each site (mean + SE).

Average blade lengths at Salmon Beach were significantly longer than those at Squaxin Island ($p < 0.001$) and Day Island (July: $p = 0.02$; August: $p < 0.001$) for months

where data is available (Table 9, Fig. 7). In addition, while average blade length was lower in September than July, the decline was not significant.

Temperature

Monthly average integrated water column temperatures differed significantly between sites during the 2018 growing season (Welch's ANOVA, $F_{19,474.42} = 34007$, $p < 0.001$). A Games-Howell post hoc test was used to determine differences between sites during each month. Temperature trends at all sites monitored showed predictable seasonal increases during the beginning of the summer, peaking near the summer solstice before declining in late summer (Table 3, Fig. 8). Average monthly temperatures increased along a north to south gradient with Salmon Beach having significantly lower temperatures than all other sites in June (Table 3, $p < 0.001$ for all pairwise comparisons). Squaxin Island was exposed to significantly higher temperatures than all other sites every month during the growing season (Table 3, $p < 0.001$ for all pairwise comparisons). Waters at Devil's Head were significantly warmer than at Day Island for all months during the growing season ($p < 0.001$) except July (Table 3).

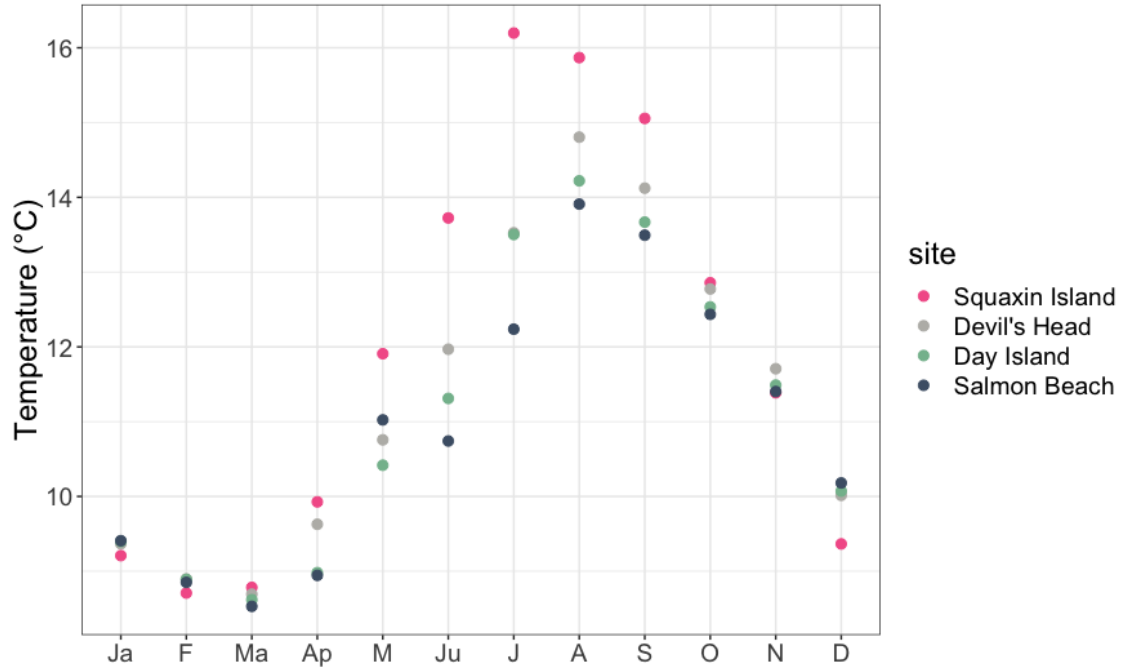


Figure 8: Average monthly integrated water column temperatures

Table 3. Temperatures (°C) as a function of survey month and site with Games-Howell pairwise post hoc differences*: Sample size, mean, standard deviation and maximum integrated water column

		<i>n</i>	<i>M</i>	<i>SD</i>	<i>MAX</i>	1	2	3
May	1. Squaxin Island	91	11.91	0.39	13.30			
	2. Devil's Head	91	10.76	0.24	11.51	-1.15		
	3. Day Island	89	10.42	0.36	11.18	-1.49	-0.34	
	4. Salmon Beach	83	11.02	0.34	11.55	-0.88	0.27	0.61
June	1. Squaxin Island	79	13.72	0.37	14.58			
	2. Devil's Head	73	11.97	0.42	13.47	-1.76		
	3. Day Island	88	11.31	0.16	11.76	-2.41	-0.66	
	4. Salmon Beach	81	10.74	0.06	10.88	-2.98	-1.23	-0.57
July	1. Squaxin Island	69	16.20	0.05	16.35			
	2. Devil's Head	91	13.53	0.22	14.02	-2.67		
	3. Day Island	88	13.50	0.33	14.01	-2.69	-0.02**	
	4. Salmon Beach	104	12.24	0.05	12.44	-3.96	-1.29	-1.27
August	1. Squaxin Island	81	15.87	0.11	15.97			
	2. Devil's Head	75	14.80	0.29	15.16	-1.06		
	3. Day Island	68	14.22	0.06	14.37	-1.65	-0.58	
	4. Salmon Beach	113	13.91	0.06	14.05	-1.96	-0.90	-0.31
September	1. Squaxin Island	52	15.06	0.03	15.11			
	2. Devil's Head	25	14.12	0.05	14.28	-0.93		
	3. Day Island	60	13.67	0.03	13.74	-1.39	-0.45	
	4. Salmon Beach	25	13.49	0.03	13.58	-1.56	-0.63	-0.18

* All pairwise comparisons $p < 0.001$

** Non-significant difference

P. producta densities

Field observations of crab density were marked by extremely high variation (Table 4, Fig. 9). Results from a Welch's ANOVA showed significant differences in crab density between sites ($F_{2,113.67} = 27.7, p < 0.001$). A Games-Howell post hoc tests showed significantly lower densities at Salmon Beach ($M = 0.03$ crabs m^{-2} , $SE = 0.01$) than Squaxin Island ($M = 0.36$ crabs m^{-2} , $SE = 0.11, p < 0.001$) and Day Island ($M = 0.38$ crabs m^{-2} , $SE = 0.05, p = 0.01$, Table 4). Average summer crab densities did not differ significantly between Squaxin Island and Day Island.

Crab densities also showed distinct trajectories at each site through time, and the seasonal patterns were different at each site (Table 10, Fig. 10). Highest recorded crab densities peaked at Squaxin Island during July ($M = 0.84$ crabs m^{-2} , $SE = 0.32$) and then declined in August ($M = 0.51$ crabs m^{-2} , $SE = 0.23$). In contrast, at Day Island crab densities steadily increased until September ($M = 0.75$ crabs m^{-2} , $SE = 0.11$, Table 10). Salmon beach had the lowest observed crab densities. However, all densities per m^2 were low rarely exceeding one crab per m^2 (Table 10, Fig. 10).

Table 4. Crab densities as a function of site with Games-Howell pairwise post hoc test p-values: Sample size, mean, standard deviation, standard error

Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	Games-Howell post hoc p-value	
					1	2
1. Squaxin Island	64	0.36	0.86	0.11		
2. Day Island	108	0.38	0.50	0.05	0.98	
3. Salmon Beach	50	0.03	0.10	0.01	0.01	5.73e10 ⁻¹⁰

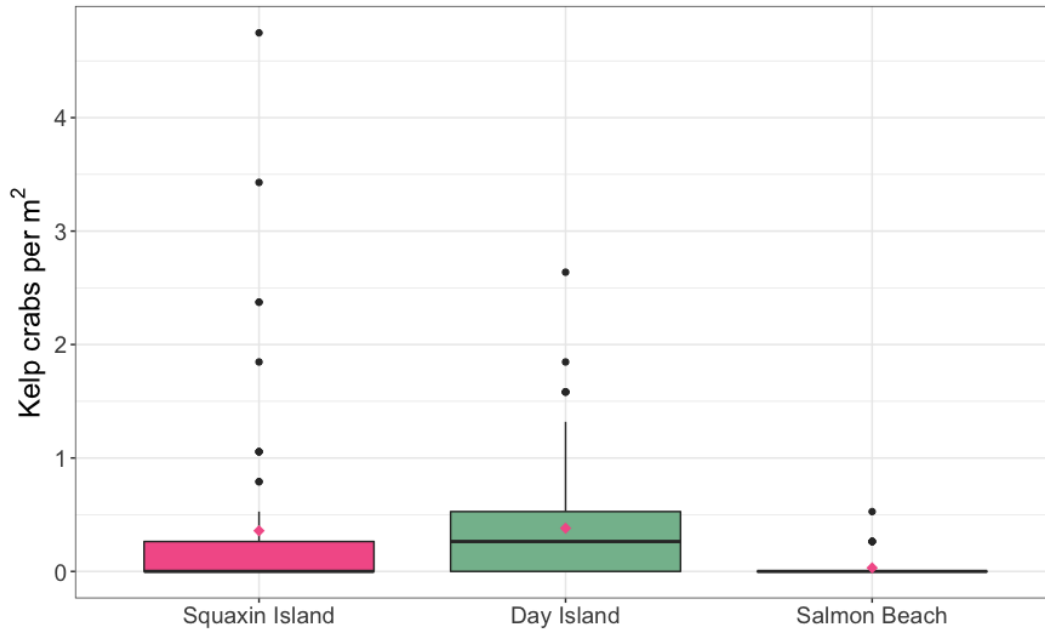


Figure 9: Crab densities at each site (black line = median, pink diamond = mean)

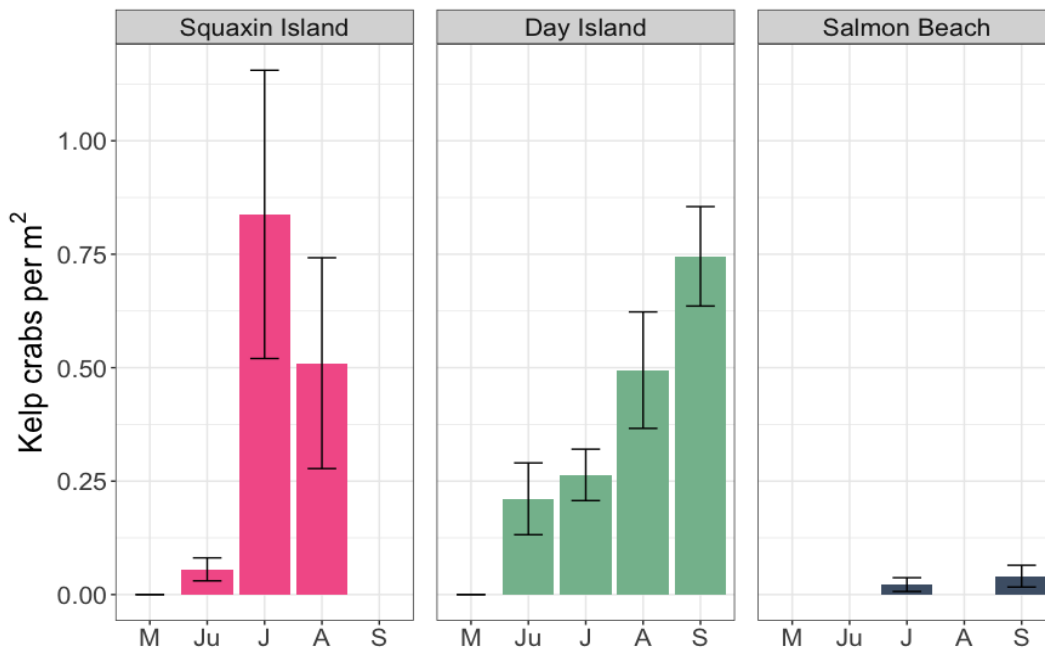


Figure 10: Average monthly crab densities at each site (mean \pm SE).

Nitrate Concentrations

Observations of nitrate concentration (μM) during the growing season documented declines in nitrate availability at Squaxin Island over the course of the summer while nitrate availability remained relatively consistent at the remaining three sites (Table 11, Fig. 12). Nitrate availability differed significantly as a function of site (one-way ANOVA, $F_{3, 22} = 43.13$, $p < 0.001$) with average seasonal nitrate concentrations significantly lower at Squaxin Island ($M = 3.43 \mu\text{M}$, $SE = 0.89$, $p < 0.001$ for all pairwise comparisons) than all other sites as determined by a Tukey's post hoc test (Table 5, Fig. 11). Nitrate concentrations at Squaxin were consistently below $5 \mu\text{M}$ from June until September in both surface waters (-0.25 m MLLW) and at a depth of -4 m MLLW ; highest growing season nitrate concentrations were observed in May at -4 m MLLW ($7.9 \mu\text{M}$) however, surface nitrate concentrations were still below $5 \mu\text{M}$ (Table 11, Fig. 12). At all other sites, months and depths nitrate concentrations were above $10 \mu\text{M}$ with the exception of Devil's Head in June where concentrations dipped to $8.8 \mu\text{M}$ at a depth of -4 m MLLW (Table 11, Fig. 12).

Table 5. Nitrate concentrations as a function of site: Sample size, mean standard deviation, standard error and 95% confidence interval

Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	<i>95% CI</i>
1. Squaxin Island	8	3.43	2.50	0.89	1.70 – 5.17
2. Devil's Head	8	11.93	1.57	0.55	10.84 – 13.02
3. Day Island	6	13.63	2.04	0.83	12.00 – 15.26
4. Salmon Beach	4	13.81	1.31	0.66	12.52 – 15.10

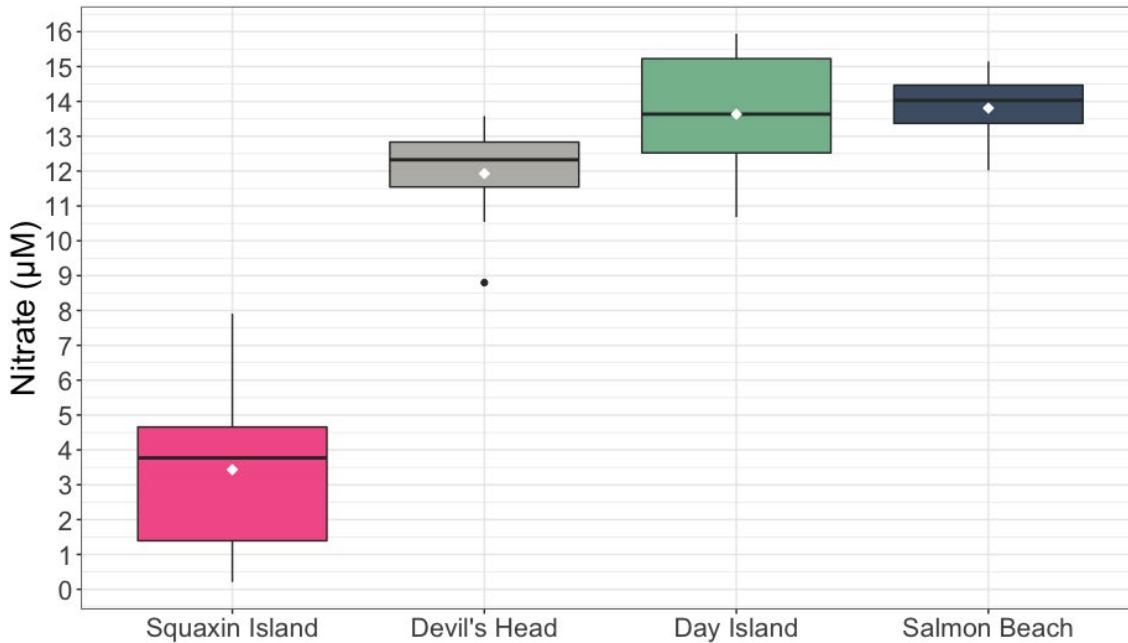


Figure 11: Growing season (May–September) water nitrate concentrations at study sites (black line = median, white diamond = mean).

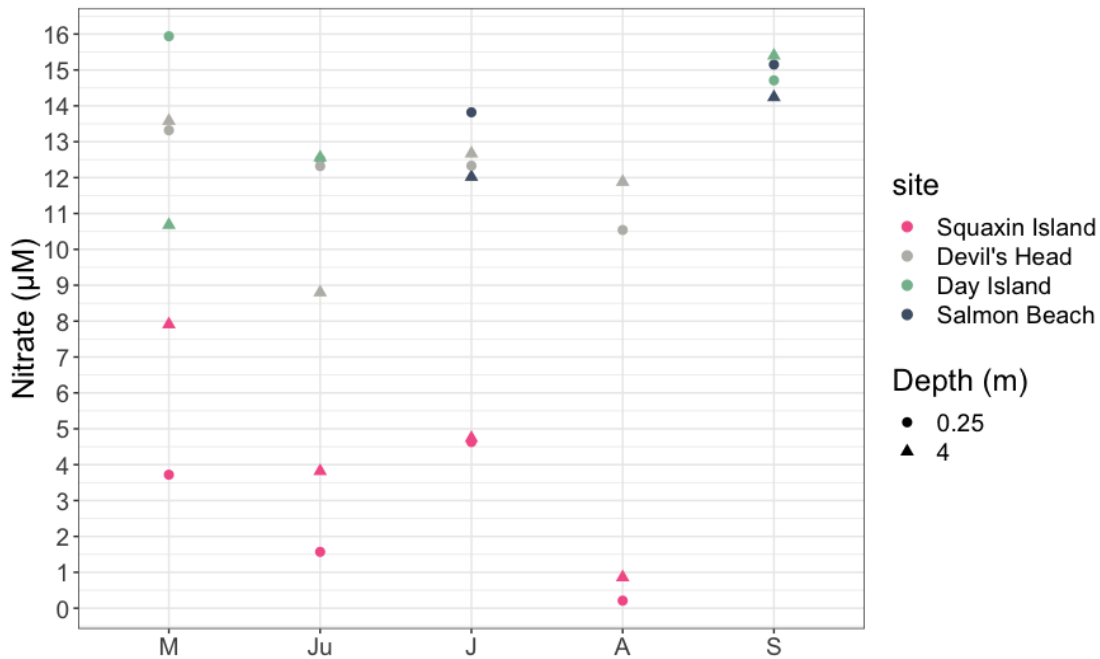


Figure 12: Monthly growing season nitrate concentrations (μM) at two depths (0.25 m and 4 m) at study sites.

Interactions Between Temperature, Crab Density and Blade Length

During field observations, blade length decreased as densities of *P. producta* abundances increased (Fig. 14). Observed reductions in blade length also coincided with highest recorded temperatures at both Squaxin Island and Day Island (Fig. 13). The combination of these two common stressors was negatively correlated with lengths, together explaining approximately 20 percent of observed variations in blade length ($F_{4,165} = 11.09$, $p < 0.01$, Table 6). Temperatures above ~ 12 °C were associated with significant decreases in blade length (Fig. 13). Crab density was also negatively correlated with blade length; however this may be due to the small number of high crab densities observed and large variation in blade lengths associated with low crab densities (Fig. 14).

Table 6. Nereocystis blade length, temperature and crab densities at all sites: Multiple linear-regression with quadratic polynomial treatment of temperature, and interaction between crab densities and average temperature

	<i>B</i>	<i>SE B</i>	<i>t</i>	<i>p</i>
(Intercept)	-12.90	4.61	-2.80	0.006
Mean Temperature	2.50	0.71	3.56	0.001
Mean Temperature ²	-0.10	0.03	-3.71	0.0003
Crab density (m ⁻²)	-2.69	1.24	-2.16	0.03
Crab density : Mean temperature	0.15	0.08	1.77	0.08

$F(4, 165) = 11.09$, $Adj. R^2 = 0.19$, $p = 5.46 e^{-8}$

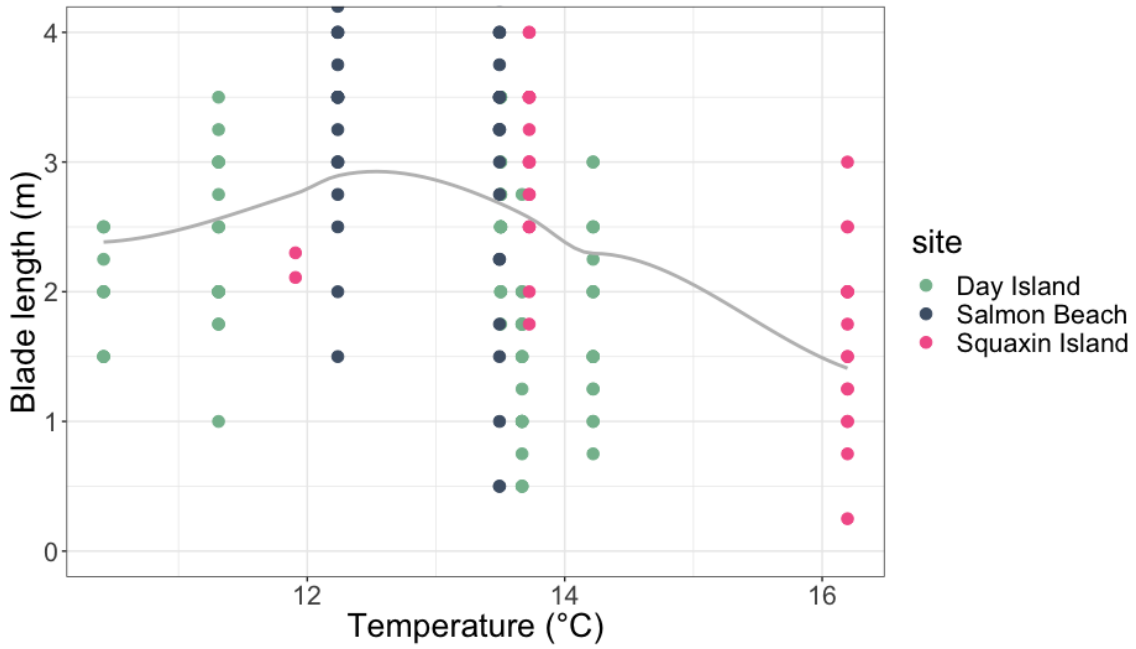


Figure 13: Blade length as a function of water temperature with regression trend line

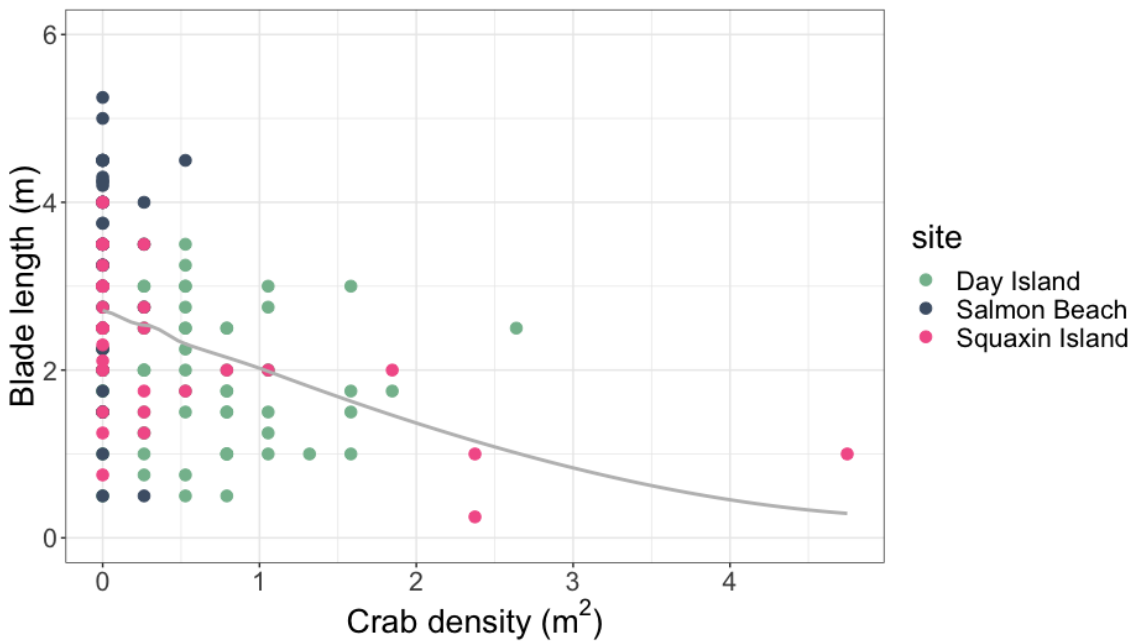


Figure 14: Blade length as a function of crab densities with regression trend line

Distinct differences in blade length, crab densities and water temperature between all sites included in the study suggested sites specific differences influencing *Nereocystis* blade length at all sites. As a result, blade lengths at each site were modeled as a function of water temperature and crab densities at each site individually. Of the three models, water temperature and crab densities only explained a significant amount of the variation measured in blade length at Squaxin Island (Table 17). As with the all-site inclusive model, the Squaxin Island regression showed a significant correlation between high temperatures and decreases in blade length, however no significant correlation was demonstrated between crab densities and blade length (Table 7, Fig.15).

Table 7. *Nereocystis* blade length, temperature and crab densities at Squaxin Island: Multiple linear-regression with quadratic polynomial treatment of temperature, and interaction between crab densities and average temperature

	<i>B</i>	<i>SE B</i>	<i>t</i>	<i>p</i>
		14.7		
(Intercept)	-43.39	6	-2.94	0.006
Mean Temperature	6.70	2.04	3.29	0.003
Mean Temperature ²	-0.24	0.07	-3.45	0.002
Crab density (m ⁻²)	-11.49	8.80	-1.31	0.20
Crab density : Mean temperature	0.69	0.54	1.276	0.21

$F(4, 31) = 12.13, R^2 = 0.56, p = 4.75 e^{-6}$

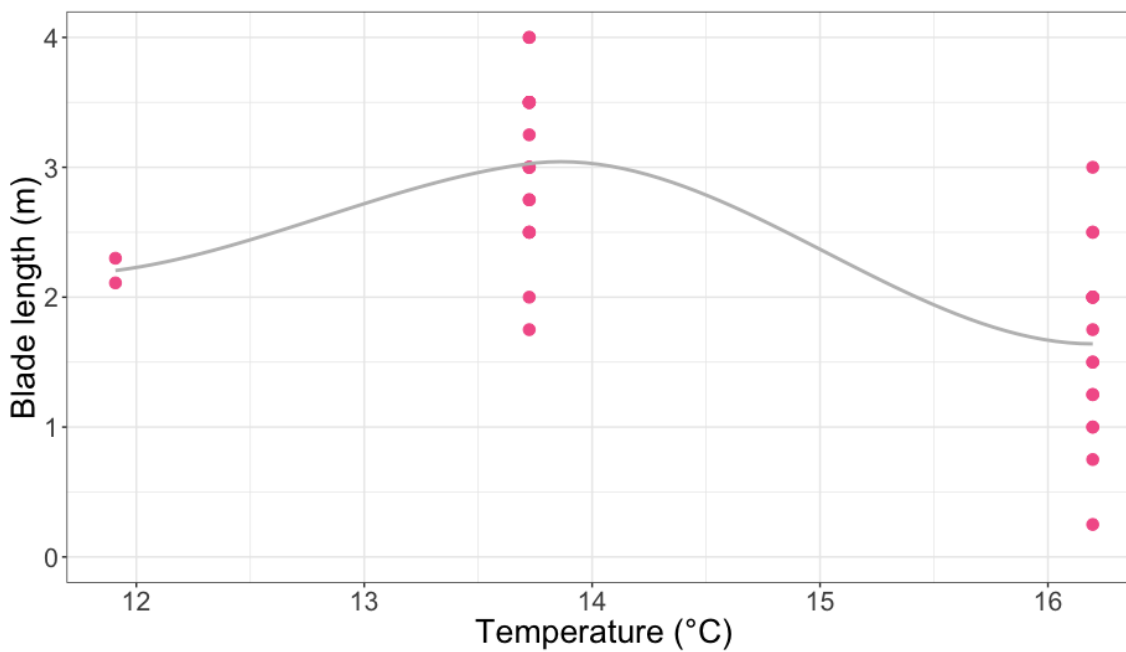


Figure 15: Squaxin Island blade length as a function of temperature with trend line of predicted values from regression analysis.

DISCUSSION

Losses to kelp forest canopies and serious negative impacts to individual plant health in the face of temperature, grazing and nutrient stress are well documented in the literature; *Nereocystis* forest canopies in the SPS appear to be no different. High temperatures and *P. producta* presence were correlated with decreases in blade length in this study. Furthermore, the kelp canopy exposed to the highest stress, Squaxin Island, also had the lowest canopy density of any of the forests monitored during this study, with the exception of Devil's Head—which never formed a floating canopy during the summer of 2018.

Plant and bed condition

Salmon Beach stands out as the healthiest forest in the SPS. While plant densities at Salmon Beach were similar to those observed at Day Island, the Salmon Beach forest had the highest densities of *Nereocystis*, and blade length was longest throughout the growing season (Table 6). Additional monitoring conducted by the Washington Department of Natural Resources (WA-DNR) also found that Salmon Beach *Nereocystis* had significantly larger bulbs and less physical damage than plants at Squaxin Island (Berry et al. 2019).

Previous observations of SPS *Nereocystis* canopies document declines in overall blade growth rates during the late summer and are similar to measured decreases in total blade length observed in this study at Day Island and Squaxin Island (Maxell & Miller 1996). Like other photosynthetic autotrophs, seaweeds rely on environmental cues for the timing of biological processes and it is possible that observed declines to blade length

during the late season may be tied to such an environmental trigger. However, studies into blade senescence in *Macrocystis* found that biological cues tied to tissue age were better at explaining rates of blade loss than environmental cues (Rodriguez et al. 2013).

Considering that *Nereocystis* is an annual species that senesces in the autumn, it is possible that reduced blade lengths in late summer are merely the product of normal annual cycles, however no research to date has been done on the mechanisms associated with *Nereocystis* tissue senescence and growth rates. Regardless, late summer blade length differed by site along a clear north to south gradient suggesting that differences in environmental conditions and biotic interactions may explain increased blade tissue loss in more southerly regions.

Environmental and biotic stressors

Average water column temperatures at the Squaxin Island forest remained above 15 °C from June until October (Table 6). In contrast, temperatures at Day Island and Salmon Beach never rose above 14.2 °C during the summer. Temperatures observed at all sites in this study were below the generally agree upon stress threshold of 17 °C. However, 25 percent of *Nereocystis* sporophytes held in artificial flumes at 15.9 °C died after one month due to an inability to recover from physical damage, suggesting reduced resiliency at temperatures near 16 °C (Tera Corp. 1982). Furthermore, concurrent monitoring of surface water temperatures within the Squaxin Island canopy itself during 2018 found a temperature gradient spanning 17 °C to 20 °C – well in the range known to significantly impact *Nereocystis* sporophyte resiliency (Berry et al. 2019).

High temperatures at Squaxin Island coincided with average nitrate concentrations below 5 μM from June to August (Table 11). In contrast, nitrate concentrations at Day Island and Salmon Beach never dropped lower than 12.5 μM . Limiting nitrogen thresholds for *Nereocystis* are unknown but *Macrocystis* requires 1 to 3 μM of total inorganic nitrogen to maintain healthy growth (Schiel & Foster 2015). Nitrate concentrations at Squaxin dipped below 1 μM only in August ($M = 0.54 \mu\text{mol}$, $0.25 \text{ m} = 0.21 \mu\text{mol}$, $4 \text{ m} = 0.86 \mu\text{mol}$, Table 11). However, Squaxin Island water temperatures remained above 15 $^{\circ}\text{C}$ from July through September and declines in *Nereocystis* photosynthetic performance at temperatures above 15 $^{\circ}\text{C}$ in conjunction with low nitrate concentrations have been documented in the literature (Wheeler et al. 1984). Furthermore, declines in Chilean *Macrocystis* forests have been observed at temperatures above 16 $^{\circ}\text{C}$ even when nitrogen concentrations are greater than 3 μM (Schiel & Foster 2015).

While this may be interpreted to mean that the Squaxin Island *Nereocystis* forest suffers from a lack of nitrate, it is more likely that seasonal algal blooms depleted summer water nitrate concentrations as has been documented in other regions of the Puget Sound (Khangaonkar et al. 2018). Surface water nutrients concentrations (top 12 m of water column) are only a fraction of concentrations encountered in deeper, oceanic inflows and nutrient transport to shallow water is a slow process (Khangaonkar et al. 2018). Instead, summer surface water nutrient concentrations are dominated by waste water treatment effluent (81 percent of summer time land based nitrogen inputs to surface waters; Mohamedali et al. 2011). Such artificially high nutrient levels may increase the frequency and severity of summer algal blooms, hogging nutrients that would otherwise be used by macroalgal communities.

As with temperature and nutrient stress, grazing stress was significantly more intense at Squaxin Island than other sites. Average *P. producta* densities at Squaxin Island peaked in July and declined sharply in August in tandem with the total loss of vegetative blades on all plants surveyed that month. Crab density at Day Island continued to increase during the entire growing season while blade length declined. In contrast, crab densities at Salmon Beach were significantly lower than at Squaxin Island and Day Island and blade length did not decline significantly in the late summer.

High temperatures and crab densities were correlated with decreases in blade length. However, low crab densities coincided with a large range of blade lengths and instances of high crab densities were relatively rare in this survey. Additionally, the negative correlation between high temperatures and blade length may also be explained by normal seasonal trends in *Nereocystis* blade growth which decline in late summer when temperatures are the highest.

Regardless, environmental conditions (temperature and nutrient concentrations) at Salmon Beach and Day Island were similar and yet Day Island plants experienced significant declines in blade length during the late summer while Salmon Beach plants did not. Observed reductions in blade length declines at Day Island coincided with increasing crab densities, suggesting that grazing pressure may cause significant damage to *Nereocystis* blade length in the SPS.

The low nutrients, high temperatures and abundance of kelp crabs make the continued persistence of the Squaxin Island forest surprising. Investigations into *Nereocystis* genetics in the Salish Sea revealed that *Nereocystis* allelic diversity decreases as one moves south from Admiralty Inlet towards the SPS with the lowest allelic richness

found at Squaxin Island (Gierke et al. 2018). This low genetic diversity may stem from adaptation to stressful conditions, but it may also be due to inbreeding depression — Squaxin Island is southernmost *Nereocystis* forest in the Salish Sea. However, if the Squaxin Island forest is indeed specially adapted to high stress conditions, it may be possible to develop temperature resilient restoration stock for future recovery and enhancement efforts currently underway in the Puget Sound.

Devil’s Head, substrate availability and bed area

Environmental conditions at Devil’s Head were similar to Day Island in that they occupied an intermediary position between Squaxin Island and Salmon Beach but, unlike Day Island, was more similar to Squaxin Island in terms of temperature (Fig. 6, Fig. 9). Despite this, the few scattered *Nereocystis* individuals encountered on snorkel surveys in June failed to reach the surface and had all disappeared two months later.

Macroalgae requires ample hard substrate on which to anchor and remain stationary in areas of high currents (Dayton 1985). Unlike the outer coast and Western Strait of Juan de Fuca, characterized by large swaths of rocky reefs composed of consolidated bedrock substrates in the Puget Sound are dominated by unconsolidated pebble, cobbles and boulders (Ebbesmeyer et al. 1988). All plants encountered during June snorkel surveys at Devil’s Head appeared to be lodged in large stands of understory *Saccharina latissima* (sugar kelp) and substrate observed consisted almost exclusively of fine sediment. Lack of available substrate combined with the low-density of 2018 recruits may point to an interaction between total bed area and substrate availability in determining forest density and long-term persistence but requires further study.

Methods Assessment

This study relied on kayak surveys for data collection. This survey method not only allowed for monitoring of target parameters but afforded an opportunity to test the efficacy of low cost and accessible methods for use in monitoring *Nereocystis* forests with the help of citizen science initiatives. Ecological data, especially in the marine realm, can be difficult and expensive to obtain. As a result, most research relies on short-term (one to five year) studies to generalize about incredibly dynamic and complex systems (Krumhansl et al. 2016). Kelp forests, in particular, are characterized by a high degree of variation over time. Much of this short-term variation is due to local variations in environmental and biotic conditions (Krumhansl et al. 2016). However, on larger scales, variation in kelp forests follows climatological scale alterations in environmental conditions (Pfister et al. 2017). Thus, low cost, easily implemented monitoring protocols could allow for more consistent assessments of Puget Sound *Nereocystis* forests over longer time scales.

Some of the data collected during this study violated assumptions for standard statistical tests in ways that are commonly encountered during ecological studies. Specifically, both plant density data and crab counts were over-dispersed due to a large number of zeros recorded during surveys. In some cases, zero-inflated data sets reflect the reality of the study population, as in the case of rare species. However, neither *Nereocystis* nor *P. producta* were rare in SPS kelp forests. Instead, the data suggests that in future monitoring more plots need to be incorporated or that another sampling protocol, perhaps across shore transects, should be selected.

Continued monitoring

More than half of the linear extent of *Nereocystis* forests in the SPS have been lost since the 1890's (Berry, personal communication). However, reasons for declines are unknown, making conservation and recovery efforts difficult to implement effectively. Sites for conservation and restoration actions can be identified from available data but a lack of information regarding kelp distributions and stressor impacts may impede the reestablishment of persistent annual canopies. Finally, the SPS is characterized by the highest temperatures and lowest nutrient availabilities of all Puget Sound basins (Berry et al. 2019). Continued monitoring of known kelp stressors and plant condition at SPS sites has the potential to help identify priority stressors for mitigation actions and also provide insight into the response of Puget Sound *Nereocystis* forest response to climate change.

CONCLUSION

The results from this study represent a first step towards identifying key stressors driving losses in the SPS but pinpointing causes for declines will require further monitoring and study. Each major basin of the Puget Sound is characterized by different oceanographic conditions meaning that causes for declines may differ by region. Regardless, the stressors monitored in this study remain high on the list of possible causes for kelp declines in the Puget Sound and elsewhere (Steneck et al. 2002).

Low densities of adult sporophytes and rapid, early season loss of *Nereocystis* blades at Squaxin Island is consistent with much of the literature regarding kelp responses to a changing climate (Harley et al. 2012). The temperatures observed at Squaxin Island were well within the accepted upper thermal range (15 to 20 °C) for *Nereocystis*. However,

temperatures at Day Island were more similar to those at Salmon Beach and yet significant late summer losses to blade length were only observed at Day Island. This suggests the strong possibility that kelp crab activity on adult *Nereocystis* sporophytes may exert a strong influence over late season blade biomass at SPS kelp forests, especially as temperatures continue to increase. These negative impacts are likely amplified at sites, such as Squaxin Island, where additional temperature and nutrient related stress interact synergistically with grazing damage. While these results confirm the suspicions of the author and other nearshore management and restoration professionals, it is difficult to understand whether grazing pressure in the SPS has increased over time due to a lack of data.

Nereocystis forests in the Puget Sound form critical habitats that provide foundations for food-webs that support healthy populations rockfish, salmon and orca. Given the recent documented *Nereocystis* losses in the SPS and other basins, identifying causes for canopy losses is essential for the effective future management.

Future studies should focus on investigating and monitoring key stressors in *Nereocystis* forests across multiple basins. In addition, future research would benefit by focusing on recording blade lengths of multiple blades on a single individual, monitoring blade growth rates, estimating sorus production rates and assessing individual fecundity. More detailed surveys of kelp crab abundances on a plant-by-plant basis (as opposed to per m²) may provide better understanding into the effect of crab grazing on adult *Nereocystis* sporophyte condition. Finally, pairing nutrient concentration monitoring with chlorophyll monitoring could provide insight into the effect of algal blooms on nutrient availability to large kelp species.

REFERENCES

- Ahn, Okhyun, Royann J. Petrell, and Paul J. Harrison. 1998. Ammonium and Nitrate Uptake by *Laminaria Saccharina* and *Nereocystis Luetkeana* Originating from a Salmon Sea Cage Farm. *Journal of Applied Phycology* Volume 10(4) pages 333–340.
- Amsler, C D, and M Neushul. 1990. Diel Periodicity of Spore Release from the Kelp *Nereocystis Luetkeana* (Mertens) Postels et Ruprecht. *J. Exp. Mar. Biol. Ecol.* Volume 134(2) pages 117–127.
- Bartsch, Inka, Christian Wiencke, Kai Bischof, Cornelia M. Buchholz, Bela H. Buck, Anja Eggert, Peter Feuerpfeil, et al. 2008. The Genus *Laminaria Sensu Lato*: Recent Insights and Developments. *European Journal of Phycology* Volume 43(1) pages 1–86.
- Berry, Helen. 2017. Assessment of Floating Kelp at Squaxin Island in 2013, 2014, and 2016. Draft. Washington State Department of Natural Resources.
- Berry, Helen. 2018. Kelp Status and Trends in Puget Sound. Powerpoint Presentation presented at the Kelp Recovery Plan Workshop: Kelp Ecosystem Services, Stressors and Coverage Trends, Seattle, WA.
- Berry, Helen D., Max Calloway, and Julia Ledbetter. 2019. Bull Kelp Monitoring in South Puget Sound in 2017 and 2018. Nearshore Washington Department of Natural Resources Habitat Program Aquatic Resources Division.
- Berry, Helen D., Thomas F. Mumford Jr, and Peter Dowty. 2005. Using Historical Data to Estimate Changes in Floating Kelp (*Nereocystis Luetkeana* and *Macrocystis Integrifolia*) in Puget Sound, Washington. No page number in *Proceedings of the 2005 Puget Sound George Basin Research Conference*. Puget Sound Action Team, Olympia, Washington. Vol. 9.
- Bertness, Mark D., John F. Bruno, Brian R. Silliman, and John J. Stachowicz, eds. 2014. *Marine Community Ecology and Conservation*. Sinauer Associates, Inc.
- Bertocci, I., R. Araújo, P. Oliveira, and I. Sousa-Pinto. 2015. REVIEW: Potential Effects of Kelp Species on Local Fisheries. Edited by Henrik Österblom. *Journal of Applied Ecology* Volume 52(5) pages 1216–26.
- Bishop, Emily. n.d. A Kayak-Based Survey Protocol for Bull Kelp in Puget Sound, 26.
- Carney, Laura T., and Matthew S. Edwards. 2006. Cryptic Processes in the Sea: A Review of Delayed Development in The Microscopic Life Stages of Marine Macroalgae. *Algae* Volume 21(2) pages 161–168.

- Crain, Caitlin Mullan, Kristy Kroeker, and Benjamin S. Halpern. 2008. Interactive and Cumulative Effects of Multiple Human Stressors in Marine Systems. *Ecology Letters* Volume 11(12) pages 1304–15.
- Dayton, Paul K. 1985. Ecology of Kelp Communities. *Annual Review of Ecology and Systematics* Volume 16(1) pages 215–245.
- Dayton, Paul K., Mia J. Tegner, Paul E. Parnell, and Peter B. Edwards. 1992. Temporal and Spatial Patterns of Disturbance and Recovery in a Kelp Forest Community. *Ecological Monographs* Volume 62(3) pages 421–45.
- Dobkowski, Katie. 2017. The Role of Kelp Crabs as Consumers in Bull Kelp Forests—Evidence from Laboratory Feeding Trials and Field Enclosures. *PeerJ* Volume 5(May) pages e3372.
- Dobkowski, Katie A., K. Darby Flanagan, and Jessica R. Nordstrom. 2019. Factors Influencing Recruitment and Appearance of Bull Kelp, *Nereocystis Luetkeana* (Phylum Ochrophyta). Edited by A. Buschmann. *Journal of Phycology* Volume 55(1) pages 236–44.
- Druehl, Louis D., and Bridgette E. Clarkston. 2016. *Pacific Seaweeds: A Guide to Common Seaweeds of the West Coast*. Second. BC Canada: Harbour Publishing.
- Duggins, David, James E. Eckman, Christopher E. Siddon, and Terrie Klinger. 2001. Interactive Roles of Mesograzers and Current Flow in Survival of Kelps. *Marine Ecology Progress Series* Volume 223 pages 143–155.
- Duggins, David O. 1980. Kelp Beds and Sea Otters: An Experimental Approach. *Ecology* Volume 61(3) pages 447–53.
- Ebbesmeyer, Curtis C., Jack Q. Word, and Clifford A. Barnes. 1988. Puget Sound: A Fjord System Homogenized with Water Recycled. No page number *in* *Hydrodynamics of Estuaries*, edited by Bjorn Kjerfve. CRC Press.
- Edwards, Matthew S. 2000. The Role of Alternate Life-History Stages of a Marine Macroalga: A Seed Bank Analogue? *Ecology* Volume 81(9) pages 2404.
- Gabrielson, P.W., S.C Linstrom, and C.J. O’Kelly. 2012. *Keys to the Seaweeds and Seagrasses of Southeast Alaska, British Columbia, Washington and Oregon*. Phycological Contribution No. 8, PhycoID, Hillsborough, NC. Iv + 192 pp.
- Gaylord, Brian, Daniel C. Reed, Peter T. Raimondi, Libe Washburn, and Stephen R. McLean. 2002. A Physically Based Model of Macroalgal Spore Dispersal in the Wave and Current-Dominated Nearshore. *Ecology* Volume 83(5) pages 1239–51.

- Gegzna, Vilmantas. 2018. *biostat: Routines for Basic (Bio)Statistics* (<https://gegznv.github.io/biostat/> version R package version 0.0.18).
- Gelfenbaum, Guy, Tom Mumford, Jim Brennan, Harvey Case, Megan Dethier, Kurt Fresh, Fred Goetz, et al. 2006. *Coastal Habitats in Puget Sound: A Research Plan in Support of the Puget Sound Nearshore Partnership*. Technical Report 2006-1. Puget Sound Nearshore Partnership.
- Gierke, Lily, Bobby San Miguel, Tom Mumford, and Filipe Alberto. 2018. Genetic Structure of the Bull-Kelp *Nereocystis Luetkeana* in the Salish Sea. presented at the Salish Sea Ecosystem Conference, Seattle, WA, April 6.
- Harley, Christopher D. G., Kathryn M. Anderson, Kyle W. Demes, Jennifer P. Jorve, Rebecca L. Kordas, Theraesa A. Coyle, and Michael H. Graham. 2012. Effects of climate change on global seaweed communities. *Journal of Phycology* Volume 48(5) pages 1064–78.
- Hines, Anson H. 1982. Coexistence in a Kelp Forest: Size, Population Dynamics, and Resource Partitioning in a Guild of Spider Crabs (*Brachyura*, *Majidae*). *Ecological Monographs* Volume 52(2) pages 179–98.
- Hurd, C.L., P.J. Harrison, K. Bischof, and C.S. Lobban. 2014. *Seaweed ecology and physiology*. Second. Cambridge University Press.
- Khangaonkar, Tarang, Adi Nugraha, Wenwei Xu, Wen Long, Laura Bianucci, Anise Ahmed, Teizeen Mohamedali, and Greg Pelletier. 2018. Analysis of Hypoxia and Sensitivity to Nutrient Pollution in Salish Sea. *Journal of Geophysical Research: Oceans* Volume 123(7) pages 4735–61.
- Krumhansl, Kira A., Jean-Sébastien Lauzon-Guay, and Robert E. Scheibling. 2014. Modeling Effects of Climate Change and Phase Shifts on Detrital Production of a Kelp Bed. *Ecology* Volume 95(3) pages 763–74.
- Krumhansl, Kira A., Daniel K. Okamoto, Andrew Rassweiler, Mark Novak, John J. Bolton, Kyle C. Cavanaugh, Sean D. Connell, et al. 2016. Global Patterns of Kelp Forest Change over the Past Half-Century. *Proceedings of the National Academy of Sciences of the United States of America* Volume 113(48) pages 13785–90.
- Lind, Alyssa C., and Brenda Konar. 2017. Effects of Abiotic Stressors on Kelp Early Life-History Stages. *ALGAE* Volume 32(3) pages 223–33.
- Lüning, Klaus, and Wilson Freshwater. 1988. Temperature Tolerance of Northeast Pacific Marine Algae. *Journal Of Phycology* Volume 24(3) pages 310–515.
- Mangiafico, Salvatore. 2019. *rcompanion: Functions to Support Extension Education Program Evaluation* (version R package version 2.0.10).

- Maxell, B. A., and K. A. Miller. 1996. Demographic Studies of the Annual Kelps *Nereocystis Luetkeana* and *Costaria Costata* (Laminariales, Phaeophyta) in Puget Sound, Washington. *Botanica Marina* Volume 39(1–6) .
- Mohamedali, T., M. Roberts, B. Sackmann, and A. Kolosseus. 2011. Puget Sound Dissolved Oxygen Model Nutrient Load Summary for 1999-2008. 11-03–057. Washington State Department of Ecology.
- Mumford, Thomas F. 2007. Kelp and Eelgrass in Puget Sound: Fort Belvoir, VA: Defense Technical Information Center.
- National Marine Fisheries Service. 2017. Rockfish Recovery Plan: Puget Sound / Georgia Basin Yelloweye Rockfish (*Sebastes Ruberrimus*) and Bocaccio (*Sebastes Paucispinis*). Seattle, WA: National Marine Fisheries Service.
- Palmer-McGee, Casey. 2019. A Decade of Dissaperance: Kelp Canopies in Samish Traditional Territory. Web site accessed March 19, 2019.
- Pfister, Catherine A., Helen D. Berry, and Thomas Mumford. 2017. The Dynamics of Kelp Forests in the Northeast Pacific Ocean and the Relationship with Environmental Drivers. Edited by A. Randall Hughes. *Journal of Ecology*, December.
- Pfister, Catherine A., and Samuel P. Betcher. 2017. Climate Drivers and Animal Host Use Determine Kelp Performance over Decadal Scales in the Kelp *Pleurophycus Gardneri* (Laminariales, Phaeophyceae). *Journal of Phycology*.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
- Rodriguez, Gabriel E., Andrew Rassweiler, Daniel C. Reed, and Sally J. Holbrook. 2013. The Importance of Progressive Senescence in the Biomass Dynamics of Giant Kelp (*Macrocystis Pyrifera*). *Ecological Society of America*.
- Rothäusler, Eva, Iván Gómez, Iván A. Hinojosa, Ulf Karsten, Fadia Tala, and Martin Thiel. 2009. Effect of Temperature and Grazing on Growth of *Macrocystis* Spp. (Phaeophyceae) along a Latitudinal Gradient. *Journal of Phycology* Volume 45(3) pages 547–59.
- Schiel, David R., and Michael S. Foster. 2006. The Population Biology of Large Brown Seaweeds: Ecological Consequences of Multiphase Life Histories in Dynamic Coastal Environments. *Annu. Rev. Ecol. Evol. Syst.* Volume 37pages 343–372.
- . 2015. *The Biology and Ecology of Giant Kelp Forests*. Oakland, California: University of California Press.

- Schiel, David R., John R. Steinbeck, and Michael S. Foster. 2004. Ten Years of Induced Ocean Warming Causes Comprehensive Changes in Marine Benthic Communities. *Ecology* Volume 85(7) pages 1833–39.
- Schmider, Emanuel, Matthias Ziegler, Erik Danay, Luzi Beyer, and Markus Bühner. 2010. Is It Really Robust?: Reinvestigating the Robustness of ANOVA Against Violations of the Normal Distribution Assumption. *Methodology* Volume 6(4) pages 147–51.
- Springer, Yuri P., Cynthia G. Hays, Mark H. Carr, and Megan R. Mackey. 2010. Toward Ecosystem-Based Management of Marine Macroalgae—The Bull Kelp, *Nereocystis Luetkeana*. *Oceanography and Marine Biology* Volume 48pages 1.
- Steneck, Robert S., Michael H. Graham, Bruce J. Bourque, Debbie Corbett, Jon M. Erlandson, James A. Estes, and Mia J. Tegner. 2002. Kelp Forest Ecosystems: Biodiversity, Stability, Resilience and Future. *Environmental Conservation* Volume 29(04) .
- Suskiewicz, Matthew S. 2010. Effects of Competition and Dispersal on the Recruitment of the Annual Kelp *Nereocystis Luetkeana*. Masters Thesis, San José, California, U.S.A.: San José State University.
- Swanson, A. K., and L. D. Druehl. 2000. Differential Meiospore Size and Tolerance of Ultraviolet Light Stress within and among Kelp Species along a Depth Gradient. *Marine Biology* Volume 136(4) pages 657–64.
- Tera Corp. 1982. Compendium of Thermal Effects Laboratory Studies: Diablo Canyon Power Plant. Volume 1. Tera Corporation.
- Vadas, R L. 1972. Ecological Implications of Culture Studies on *Nereocystis Luetkeana*. *Journal of Phycology*, no. 8pages 196–203.
- Wernberg, Thomas, Mads S. Thomsen, Fernando Tuya, Gary A. Kendrick, Peter A. Staehr, and Benjamin D. Toohey. 2010. Decreasing Resilience of Kelp Beds along a Latitudinal Temperature Gradient: Potential Implications for a Warmer Future: Climate and Resilience of Kelp Beds. *Ecology Letters* Volume 13(6) pages 685–94.
- Wernberg, Thomas, Mathew A. Vanderklift, Jason How, and Paul S. Lavery. 2006. Export of Detached Macroalgae from Reefs to Adjacent Seagrass Beds. *Oecologia* Volume 147(4) pages 692–701.
- Wheeler, W. N., R. G. Smith, and L. M. Srivastava. 1984. Seasonal Photosynthetic Performance of *Nereocystis Luetkeana*. *Canadian Journal of Botany* Volume 62(4) pages 664–70.

- Wiencke, Christian, Michael Y. Roleda, Ansgar Gruber, Margaret N. Clayton, and Kai Bischof. 2006. Susceptibility of Zoospores to UV Radiation Determines Upper Depth Distribution Limit of Arctic Kelps: Evidence through Field Experiments. *Journal of Ecology* Volume 94(2) pages 455–63.
- Williams, G.D., R.M. Thom, J.E. Starkes, J.S. Brennan, J. P. Houghton, D. Woodruff, P.L. Striplin, M. Miller, M. Pedersen, A. Skillman, R. Kropp, A. Borde, C. Freeland, K., and McArthur, V. Fagerness, S. Blanton, and L. Blackmore. 2001. Reconnaissance Assessment of the State of the Nearshore Ecosystem: Eastern Shore of Central Puget Sound, Including Vashon and Maury Islands (WRIAs 8 and 9). Seattle, WA: King County Department of Natural Resources.
- Zeileis, Achim, and Torsten Hothorn. 2002. Diagnostic Checking in Regression Relationships (<https://CRAN.R-project.org/doc/Rnews/> version).

APPENDIX

Table 8. Plant density per square meter as a function of site and survey date: sample size, mean, standard deviation, standard error, median, first quartile, third quartile and interquartile range of

Month	Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	<i>MED</i>	<i>Q1</i>	<i>Q3</i>	<i>IQR</i>
May	Squaxin Island	13	0.87	0.74	0.21	0.79	0.26	1.06	0.79
	Day Island	10	5.80	3.67	1.16	4.75	3.49	9.23	5.74
	Salmon Beach	--	--	--	--	--	--	--	--
Jun.	Squaxin Island	19	0.67	0.98	0.23	0.26	0.00	0.92	0.92
	Day Island	25	2.35	2.50	0.50	1.06	0.53	3.17	2.64
	Salmon Beach	--	--	--	--	--	--	--	--
Jul.	Squaxin Island	17	0.47	0.58	0.14	0.26	0.00	0.79	0.79
	Day Island	26	2.22	2.46	0.48	1.06	0.53	3.49	2.97
	Salmon Beach	24	2.42	2.11	0.43	1.85	0.79	3.17	2.37
Aug.	Squaxin Island	15	0.30	0.37	0.10	0.00	0.00	0.53	0.53
	Day Island	24	1.68	1.72	0.35	1.06	0.46	2.31	1.85
	Salmon Beach	--	--	--	--	--	--	--	--
Sep.	Squaxin Island	--	--	--	--	--	--	--	--
	Day Island	23	1.28	1.47	0.31	0.53	0.40	1.58	1.19
	Salmon Beach	26	1.54	1.39	0.27	1.06	0.79	2.31	1.52

Table 9. Blade length (m) as a function of site and survey month: sample size, mean, standard deviation, standard error, median and 95% confidence interval

Month	Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	95% <i>CI</i>
May	Squaxin Island	2	2.21	0.13	0.10	2.02 – 2.39
	Day Island	14	2.11	0.46	0.12	1.87 – 2.35
	Salmon Beach	--	--	--	--	--
Jun.	Squaxin Island	19	2.99	0.64	0.15	2.70 – 3.27
	Day Island	17	2.38	0.65	0.16	2.07 – 2.69
	Salmon Beach	--	--	--	--	--
Jul.	Squaxin Island	17	1.69	0.73	0.18	1.35 – 2.04
	Day Island	20	2.68	0.53	0.12	2.44 – 2.91
	Salmon Beach	23	3.51	0.81	0.17	3.18 – 3.84
Aug.	Squaxin Island	--	--	--	--	--
	Day Island	18	1.83	0.69	0.16	1.52 – 2.15
	Salmon Beach	--	--	--	--	--
Sep.	Squaxin Island	--	--	--	--	--
	Day Island	20	1.34	0.59	0.13	1.08 – 1.60
	Salmon Beach	26	3.18	1.30	0.25	2.68 – 3.68

Table 10. Crab densities as a function of site and survey month: sample size, mean, standard deviation, standard error, median, first quartile, third quartile and interquartile range

Month	Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	<i>MED</i>	<i>Q1</i>	<i>Q3</i>	<i>IQR</i>
May	Squaxin Island	13	0	0	0	0	0	0	0
	Day Island	10	0	0	0	0	0	0	0
	Salmon Beach	--	--	--	--	--	--	--	--
June	Squaxin Island	19	0.06	0.11	0.03	0	0	0	0
	Day Island	25	0.21	0.40	0.08	0	0	0.26	0.26
	Salmon Beach	--	--	--	--	--	--	--	--
July	Squaxin Island	17	0.84	1.31	0.32	0.26	0	1.06	1.06
	Day Island	26	0.26	0.29	0.06	0.26	0	0.26	0.26
	Salmon Beach	24	0.02	0.07	0.02	0	0	0	0
August	Squaxin Island	15	0.51	0.90	0.23	0	0	0.66	0.66
	Day Island	24	0.49	0.63	0.13	0.26	0	0.79	0.79
	Salmon Beach	--	--	--	--	--	--	--	--
September	Squaxin Island	--	--	--	--	--	--	--	--
	Day Island	23	0.75	0.53	0.11	0.79	0.40	1.06	0.66
	Salmon Beach	26	0.04	0.12	0.02	0	0	0	0

Table 11. Nitrate concentrations (μM) as a function of survey month and depth at each site: sample size, mean, standard deviation, standard error and 95% confidence interval

Month	Site	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SE</i>	95% <i>CI</i>	Depth (m)	Nitrate
May	Squaxin Island	2	5.82	2.96	2.10	1.71 – 9.92	0.25	3.72
							4	7.91
	Devil's Head	2	13.45	0.18	0.13	13.20 – 13.70	0.25	13.32
							4	13.58
	Day Island	2	13.31	3.72	2.63	8.16 – 18.46	0.25	15.94
							4	10.68
June	Squaxin Island	2	2.70	1.59	1.13	0.49 – 4.90	0.25	1.57
							4	3.82
	Devil's Head	2	10.56	2.49	1.76	7.11 – 14.01	0.25	12.32
							4	8.80
	Day Island	2	12.54	0.04	0.03	12.49 – 12.58	0.25	12.51
							4	12.56
July	Squaxin Island	2	4.69	0.08	0.06	4.58 – 4.79	0.25	4.63
							4	4.74
	Devil's Head	2	12.50	0.24	0.17	12.17 – 12.83	0.25	12.33
							4	12.67
	Salmon Beach	2	12.92	1.27	0.90	11.16 – 14.68	0.25	13.82
							4	12.02
August	Squaxin Island	2	0.54	0.46	0.33	- 0.10 – 1.17	0.25	0.21
							4	0.86
	Devil's Head	2	11.21	0.95	0.67	9.90 – 12.52	0.25	10.54
							4	11.88
September	Day Island	2	15.06	0.49	0.35	14.38 – 15.73	0.25	14.71
							4	15.40
	Salmon Beach	2	14.70	0.64	0.46	13.80 – 15.59	0.25	15.15
							4	14.24