**Spatial patterns of animal-mediated nutrients and effects on growth of the intertidal seaweed *Fucus distichus***

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**ABSTRACT**

Seaweeds are a part of some of the most productive global ecosystems, but this productivity is constantly constrained by nutrient availability in the ocean. Nitrogen is one of the main nutrients inhibiting seaweed growth, specifically in the form of ammonium. Although ammonium is imperative for productivity, the spatial patterns of it are poorly understood. Here I investigated the spatial patterns of ammonium in Indian Arm, British Columbia, and the potential effects on seaweed. Water samples were taken at the surface level and at 6 m depth to explore the distribution of ammonium. Sea cucumber surveys were completed and compared to the observed concentrations of ammonium in seawater, as sea cucumbers are a likely source of ammonium in the Indian Arm. I also tested the effects of ammonium on early *Fucus distichus* growth and germination. Additionally, samples of a native seaweed (*F. distichus*) and an invasive seaweed (*Sargassum muticum*) were collected to determine the percent nitrogen content. I found that ammonium concentrations in seawater were homogenous at the surface and variation between sites became evident once samples at 6 m depth were taken. There was a marginally significant relationship between sea cucumber population density and the ammonium concentration of site. The probability of germination of *F. distichus* zygotes was higher with both low and high added concentrations of ammonium. The growth length of *F. distichus* zygotes was significantly higher in the high ammonium addition treatment. By demonstrating that ammonium can improve the early growth of seaweeds we reiterate the important role of organisms such as sea cucumbers to provide nutrients for primary producers. This study is an early exploration of patterns of ammonium concentrations in salt-water fjords. More studies must be done to further understand why we see the ammonium patterns that we do as ammonium is an important nutrient in early seaweed growth.

**INTRODUCTION**

Macroalgal ecosystems such as kelp forests and seaweed beds are among the most extensive and productive coastal ecosystems at a global scale (Krause-Jensen & Duarte, 2016; Duarte, 2017). These ecosystems are ecologically important as they provide critical habitats for fish, invertebrates, and other marine life (Veiga et al., 2014). Additionally, macroalgal ecosystems can enhance local biodiversity and fuel inshore food webs (Veiga et al., 2014). Coastal macrophytes (seagrass beds and macroalgae) have high rates of primary productivity as they fix and sequester ~ 50% of all carbon stored in marine sediments, while only covering 2% of the global ocean (Duarte & Cebrián, 1996; Duarte, 2017; Macreadie et al., 2019).

Primary productivity in the ocean is often limited by nutrient availability (Redfield, 1958). Limited nutrient resources are a common constraint of seaweed growth, especially since nutrient availability can be highly variable and dynamic in marine environments (Xiao et al., 2019). Nitrogen is one of the most common nutrients limiting ocean productivity and seaweed growth (Redfield, 1958; Bracken, 2004; Xiao et al., 2019). When nitrogen supply increases, diversity and productivity of seaweeds also increase (Bracken & Nielsen, 2004; Bracken, 2004, Pfister et al., 2014).

There are two main forms of nitrogen in the ocean. One is nitrate, which is predominantly sourced from coastal upwelling (Mann, 1973). Another form is ammonium, which is an animal-mediated nutrient excreted by many animals, both vertebrates (e.g., fish) and invertebrates (e.g., mussels, sea cucumbers). These organisms can increase the ambient concentrations of ammonium in the environments they inhabit (McIntyre et al., 2008; Aquilino et al., 2009; Pfister et al., 2014). Ammonium is especially important for algal species as it can be taken up directly and increases local productivity and diversity (Bracken, 2004; Bracken & Nielsen, 2004; Pfister, 2007). Marine nitrogen varies not only in form but also spatially (Pfister et al., 2014). For example, ammonium concentrations can vary with distance from shore, reflecting the organisms that are present in different areas (Pfister et al., 2014). There are few studies that examine the spatial distribution of ammonium in marine environments on any scale. By this lack of knowledge regarding ammonium distribution, we also have a poor understanding of how these patterns are influencing primary producers such as seaweeds. Regardless of spatial distribution, we are aware that ammonium can have impacts to seaweed, but we are unaware at which life stages of the seaweeds that ammonium is influencing.

This study aims to explore the spatial patterns of ammonium in Indian Arm, British Columbia (BC), Canada, and to determine the effects of ammonium on the early growth stages of a common intertidal seaweed, *Fucus distichus*. I specifically asked if the concentration of ammonium in seawater is correlated to the number of sea cucumbers in a location, as they are a possible biotic source of ammonium. Additionally, I asked if *F. distichus* early growth and germination is influenced by the concentration of ammonium in seawater. Furthermore, I investigated whether the percent nitrogen content differed between a native and an invasive species of seaweed. I used a combination of surface and subsurface water samples collection and ammonium analysis, subtidal surveys of sea cucumbers, germination, and early growth experiments of *Fucus distichus* under varying ammonium concentrations, as well as percent nitrogen content analyses to answer these questions.

**METHODS**

**Study sites and study species**

The study was conducted in Indian Arm, a salt-water fjord located north of Vancouver, British Columbia, Canada. The fjord extends 20 km north of Burrard Inlet and is commonly used for recreational purposes. Fjords typically exhibit strong stratification, with surface waters constantly moving and deeper waters having little opportunity for mixing (Stigebrandt, 2012). The hydrodynamics of fjords are well understood, but both the spatial patterns of nutrient distribution and their causes are less understood, prompting this study to investigate these patterns and their implications for seaweed growth.

*Fucus distichus* is a monoecious brown alga with a diplontic life history (Pearson & Brawley, 1996), abundant in the coastal waters of British Columbia ​​(Lamb et al., 2005). During reproduction, eggs and sperm are released into the surrounding seawater where fertilization occurs, and the newly created zygotes settle onto the substratum (Van Den Hoek et al., 1995). The zygotes grow by the extension of a rhizoid towards light, making *F. distichus* an ideal species to measure growth at the earliest life stage.

Giant California sea cucumbers (*Apostichopus californicus*) are quite common along the coastlines of British Columbia. These creatures feed on detritus on the sea floor and are constantly excreting wastes from their bodies (iNaturalist, n.d.). Within this waste is ammonium, and sea cucumbers can have high ammonium excretion rates (Yu et al., 2019). Due to their high excretion rates and prevalence within the study location, sea cucumbers are an appropriate organism to investigate the impacts of animal mediated nutrients on seaweeds.

**Sea cucumber surveys**

Sea cucumber population surveys were completed at seven sites in Indian Arm. Two divers performed 20-minute roving surveys on rocky reefs at depths of 5 to 12 m, with each diver searching for sea cucumbers in crevices and under overhangs. Each diver counted all sea cucumbers seen, ensuring that there was no double counting of individuals. The numbers reported by each diver were then summed for a total count per 20 min.

**Water sampling to measure ammonium**

Prior to field collection, all laboratory equipment and sample collection bottles were acid soaked in 5% HCl solution for at least 30 min, rinsed in distilled water, and left to air dry. The working reagents for fluorometry (sodium sulphite, borate buffer, and OPA) were prepared ahead of field sampling according to Holmes et al. (1999). At each field site, I rinsed a sterile 50 ml syringe by taking up, swirling, and discarding 20 ml of seawater. I then took another 60 ml of seawater, from which 20 ml was transferred via a luer lock filter tip to rinse the sample bottle. Once the contents were discarded, the remaining 40 ml were then added to the sample bottle and the bottle is stored in a cooler. Three samples (bottles) are collected from each site, two as replicates and one to be used for the matrix calculation. Matrix bottles were collected so that fluorometry calculations and matrix effects could be calculated based on methods by Taylor et al. (2007). One replicate came from a syringe and the matrix came from water collected in a whirlpack. The water sample described above was conducted at the surface (0 m) and by divers at 6 m depth.

Before processing samples in the lab, I created standard curve bottles with 7 known concentrations (0, 200, 400, 600, 800, 1000, 1200 µmol/L) of ammonium using distilled water. Matrix bottles from each site had 200 µL of the ammonium stock solution added to them. All collected samples and standard bottles are then brought into a dark room with red light and 10 ml of OPA were added to each sample and standard bottle. All bottles were then left overnight. The following morning, the standard curve bottles were run first (in the dark) to ensure an appropriate standard curve was created. All field samples were then run in the fluorometer, ensuring three measurements within 3 fluorometry units were taken from each sample. I calculated matrix effects based on Taylor et al. (2007) to analyse how much ammonium was present in each sample.

***Fucus distichus* early growth experiments**

I used sea water and excretion from giant California sea cucumbers from Reed Point Marina (49.291284, -122.884594) located at the base of Indian Arm to create relevant concentrations of ammonium ranging from low (1 µmol/L) and high (3 µmol/L), as well as a control with no added ammonium. I took 10L of seawater and filtered and stored it in an acid-washed container to create the water for the control treatment group. To create the water for the low ammonium treatment, I placed a 764 g (wet weight) sea cucumber in a bucket filled with 10 L of unfiltered seawater. Based on previously calculated excretion rates of sea cucumbers (unpublished data), the sea cucumber remained in the bucket for 38.5 minutes to achieve an ammonium concentration of 1 µmol/L. The resulting effluent was then filtered into an acid-washed container. Similarly, to create the high ammonium treatment water, I placed a 718 g sea cucumber (wet weight) in a bucket filled with 10 L of unfiltered seawater, and left it to incubate for 119.1 minutes to achieve a high ammonium concentration of approximately 3 µmol/L. The water was then filtered and stored in an acid-washed container in a refrigerator. Fluorometry, as previously described, was conducted to determine the concentration of ammonium in the water. The pH was also measured to ensure there was no difference between the three types of treatment seawater.

We collected *Fucus distichus* on 7 March 2022 from Boulder Island (49.311493, -122.937502), at the southern end of Indian Arm. Fertile fronds of *F. distichus* are characterized by swollen, bumpy, and slimy receptacles. Fronds were collected haphazardly from multiple individuals and pooled together in large sealable plastic bags. Fifteen full individuals (thalli) were collected, including their holdfast to be able to later differentiate between individuals. Excess water was removed from the bags and a wet paper towel was placed in each bag to prevent desiccation. The bags were stored in a cooler for transport and later placed in a refrigerator, in the dark, for three days. This process stressed the organisms, encouraging the release of gametes once placed back into a favourable environment. After three days, I removed the fronds from the refrigerator. For each treatment level, I added 120 ml of one of the corresponding seawater treatments (control, low, or high) to a large acid-washed, plastic petri dish for a total of 3 dishes. I removed three fronds (containing two receptacles) from each individual *Fucus* thallus, and placed one frond into each petri dish. This was done to control for possible variation between individual thalli. The dishes were then placed in the refrigerator for 30 min and then poured through a strainer into acid-washed beakers to separate the fronds from the newly created ‘zygote water’. The beakers were swirled, then left for a few min to allow the *Fucus* zygotes to settle on the bottom. I then placed 800 µl of ‘zygote water’ and 5 ml of the appropriate seawater effluent into a small petri dish, creating six replicates of each of the three treatments (low ammonium, high ammonium, and control; 18 dishes). Lids were placed on each dish to prevent evaporation. All dishes were in a growth chamber at 15°C with a 12-hour light-dark cycle with unidirectional fluorescent light bars. Samples were placed in a haphazard order along the light bars. Every 24 h until the end of the germination period, at 120 h, I removed the samples from the growth chamber and photographed the germinated zygotes using a dissecting microscope. When the samples were returned to the growth chamber they were placed in a haphazard order along the light bar, maintaining the same petri-dish orientation to promote growth towards the light. At 120 h, I photographed the germinated zygotes and counted the number of germinated and ungerminated zygotes. All images were then analyzed in Fiji (Version 2.3.0) to measure the growth length of the rhizoid (Schindelin et al., 2012).

**Percent nitrogen content**

Thalli of *Fucus* *distichus* and *Sargassum muticum* were collected from Boulder Island respectively on 7 March 2022 and 25 February 2022 to be analyzed for percent nitrogen content. For both species, individual organisms were collected haphazardly from their holdfast and placed individually into clean sealable plastic bags. Twelve individuals of each species were collected. Excess water was drained from each sample bag. Samples were transported in coolers until they could be frozen.

Samples were removed from the freezer to be oven-dried. For *S. muticum*, the top 10 cm of growth of each individual was removed and placed in aluminum weigh trays. For *F. distichus*, frond ends containing four receptacles were removed from each individual and placed in aluminum weigh trays. After preparing each sample, all surfaces and tools were sterilized with ethanol to prevent cross-contamination. All tissue samples were dried at 65°C for 48 h. Dried samples were then homogenized into a powder using a Wig-L-Bug®. Samples were packed in aluminum packing capsules containing between 2.000 mg to 5.000 mg of the sample. Sterilization with ethanol of all tools and surfaces was done between each sample. The packed samples were then placed into 96-well cell culture plates for transport. Samples were sent to the University of British Columbia for percent nitrogen analysis.

**Statistical analysis**

I used a linear model to assess the relationship between the ammonium concentration at 6 m depth and sea cucumber density. In the germination experiment, I scored each *F. distichus* zygote as either germinated or not 5 days after the start of the experiment. The distribution was logistic. I used a generalized linear mixed-effects model and a Tukey’s HSD post-hoc test to examine the percent of *F. distichus* zygotes that germinated at the end of the experimental period based on ammonium treatment (three levels: control, low, high). I used a binomial data distribution, and dish as a random effect to account for the multiple zygotes measured per dish. A Tukey’s HSD was performed as a post-hoc test to test for differences between ammonium treatment levels. To assess the effects of ammonium concentrations on the growth of *F. distichus,* a generalized linear mixed-effects model and a Tukey HSD test were also used. Dish was included as a random effect and the data was checked for normality. The packages lme4 (Bates et al., 2015) and emmeans (Lenth, 2021) were used.

**RESULTS**

**Spatial distribution of ammonium**

Surface water samples were taken from 12 sites throughout Indian Arm, ranging from North Croker Island to Carraholly Point (Fig. 1). At all 12 sites sampled, there were no detectable concentrations of ammonium found at the surface. Water samples were also taken at 6 m depth from Carraholly Point, Boulder Island, Twin Islands, Brighton Beach, and Best Point. The highest ammonium concentration occurred at Carraholly Point (with an average of 1.882 µmol/L) and the lowest occurred at Twin Islands (0.556 µmol/L) (Fig. 2). There was no evidence of a latitudinal gradient in ammonium concentrations at 6 m (Fig. 2).

**Ammonium concentration and sea cucumber population density**

Carraholly Point, Boulder Island, Twin Islands, Brighton Beach, and Best Point were sampled for both sea cucumber populations and ammonium levels. Sea cucumber surveys were also done at Grey Rocks Island and Jug Island (Fig. 3). The highest population density sea cucumbers were observed at Carraholly Point, with 72 sea cucumbers per 20 min, and the lowest occurred at Twin Islands, with only two sea cucumbers per 20 min. There was a positive correlation between sea cucumbers density at a site and ammonium concentration, although this effect was marginally non-significant (coefficient estimate = 0.013, se = 0.007, p = 0.099; Fig. 4).

**Early growth experiments**

The low ammonium treatment had the highest germination success of *F. distichus* and the control group had the lowest germination success (Fig. 5). The probability of successful germination of *F. distichus* zygotes was significantly higher in both ammonium addition treatments than in control conditions (Tukey tests, p < 0.0001 for both comparisons; Fig. 5). There was no significant difference in germination between the low and the high ammonium treatments (Tukey test, p = 0.39; Fig. 5).

*Fucus distichus* zygotes grew significantly longer in the high ammonium treatment than in control conditions (Tukey tests, p = 0.0172, Fig, 6). Additionally, zygotes grew significantly longer in the high ammonium treatment compared to the low ammonium treatment (Tukey tests, p = 0.0263, Fig. 6). There was no significant difference in growth length observed between the control and the low ammonium treatment.

**DISCUSSION**

Here, I was able to observe that the spatial patterns of ammonium vary between surface and subsurface water samples, and that ammonium concentrations can vary between sites when measured at a depth of 6 m. These subsurface ammonium patterns are marginally correlated to the abundance of sea cucumbers at a site. As for *F. distichus* growth, a higher addition of ammonium to seawater led to increased growth length. The percent of germinated zygotes was higher in treatments with added levels of ammonium.

This study explored the distribution of ammonium within an eastern Pacific fjord. I found homogeneity in ammonium levels at the sea surface. However, ammonium concentrations in subsurface water samples from 6 m depth were heterogeneous among sampled sites. This is likely due to the topography and oceanography of fjords: as surface waters are constantly mixed by wind, any ammonium produced in a site may be relocated away from the point of production. Fjords also have characteristic sills that prevent deeper waters from exiting the fjord (Stigebrandt, 2012). This potentially allows for some accumulation of ammonium, leading to differences in ammonium between sites below the surface.

The spatial patchiness in ammonium concentrations was weakly linked to variation in sea cucumber abundance. However, a higher sample size is needed to understand better the strength of this relationship. This result is nevertheless consistent with literature showing that organisms such as sea cucumbers can add significant amounts of ammonium to the environments they inhabit (McIntyre et al., 2008; Aquilino et al., 2009; Pfister et al., 2014). In Indian Arm as in other parts of the BC coast, California sea cucumbers are among the most abundant macroinvertebrates and they contribute substantially to animal biomass (E. Lim, personal communication, 2022). There are organisms other than sea cucumbers, such as fish, that are also important organisms regarding nutrient cycling and providing ammonium to primary producers. Here, I have used sea cucumbers as an example of a species providing ammonium provisioning in order to evaluate the effects to seaweeds, but they appear to be a quite good species at doing so.

The addition of ammonium in seawater from sea cucumbers aided in both the germination and early growth length of *Fucus distichus* zygotes. I found that low amounts of additional ammonium increased the probability of zygote germination. Moreover, slightly higher additions of ammonium result in faster zygote growth. This is an interesting finding of this study, as I demonstrated that even a slight addition in ammonium can have benefits to macroalgae. Ammonium is generally taken up by seaweeds through passive diffusion rather than active transport, which is how nitrate is integrated (Roleda & Hurd, 2019). As ammonium is easily taken up into a seaweed compared to other nitrogen sources, efficient absorption of nutrients may be a possible cause as to why germination and growth are increased with the addition of ammonium. As lower additions of ammonium were not observed to increase growth of *F. distichus* zygotes, this suggests that there may be a threshold value of much ammonium is available before we begin to see benefits to early seaweed growth.

These results suggest that increase ammonium concentrations can benefit early life stages of *F. distichus.* If so, then I predict that this bolstered early growth may allow for increased success and growth of adult seaweeds. I predict that biomass surveys may show that there is a difference in the *F. distichus* biomass at sites relative to the concentrations of ammonium in the water. If there is more ammonium in the water at hotspot sites, then this increased ammonium can increase germination and growth of *F. distichus* leading to higher biomass. Furthermore, I suspect there may be a difference between the abilities of invasive fast-growing seaweeds and native seaweeds to take up nitrogen between sites with different concentrations of ammonium. I would like to collect both *S. muticum* and *F. distichus* from a low ammonium site and a high ammonium site and see if there is variation in the percent nitrogen taken up between the species and the different sites. This may be limited to the presence of *S. muticum* at these sites, as it is not as prevalent in Indian Arm as *F. distichus* is (which is a good thing).

This study demonstrates the need to further understand the spatial variability in ammonium concentrations in seascapes as it can influence the early growth of algae, with potential repercussions for their distribution and abundance. Previous studies have demonstrated that more ammonium, and nitrogen in general, can lead to increased growth of adult seaweeds, but this study demonstrates how ammonium additions can also aid growth at the early life stages. I also highlighted the importance of organisms such as sea cucumbers, as they play an important role in providing nutrients that can bolster early seaweed growth. This reiterates the importance of maintaining animal biomass to preserve nutrient provisioning for primary producers and to maintain healthy, functioning marine ecosystems.

Map

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Figure 1. Sites of surface water samples in Indian Arm, British Columbia.

Map

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Figure 2. Ammonium concentrations at 6 m depth in Indian Arm, British Columbia.

Map

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Figure 3. The number of sea cucumbers present from surveys throughout Indian Arm.

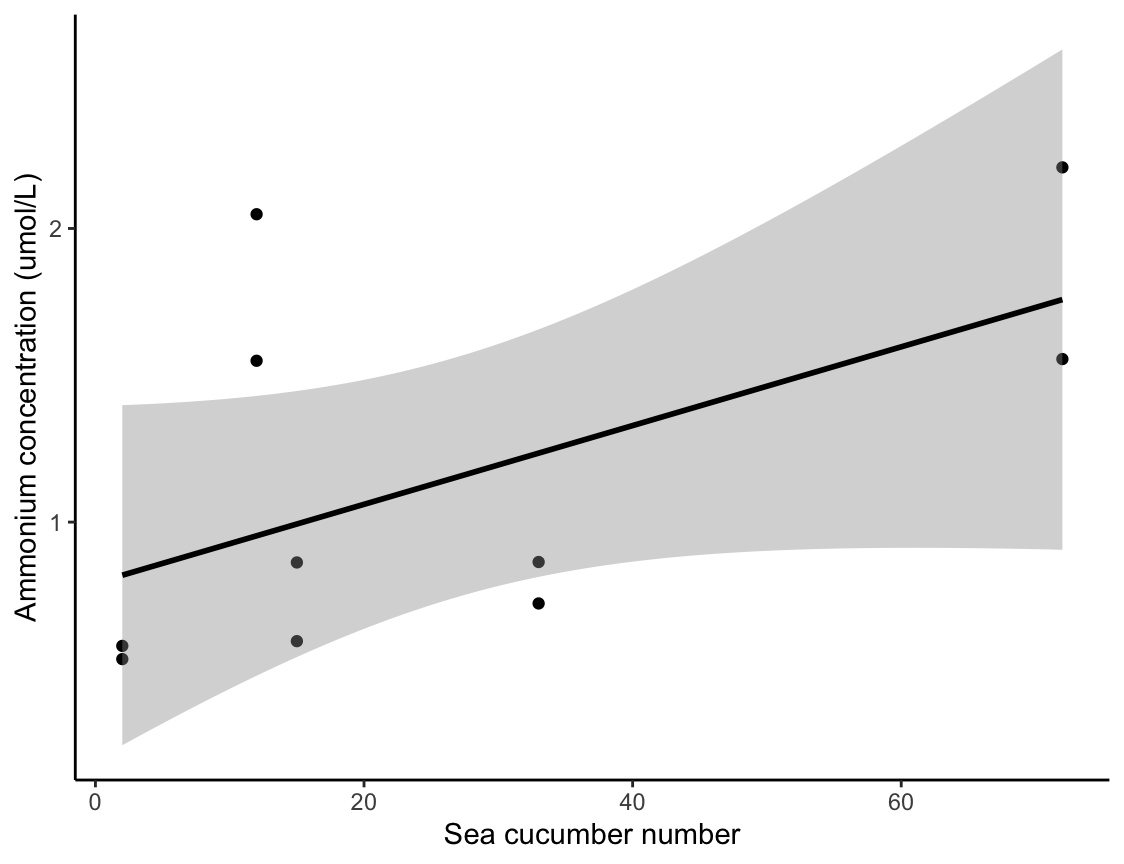


Figure 4. The relationship between sea cucumber number and the ammonium concentration measured at a site in µmol/L.

Chart, box and whisker chart

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Figure 5. The probability of germination of *Fucus distichus* zygotes in control, low, and high ammonium seawater treatments after 5 days of being in a growth chamber.

Chart, box and whisker chart

Description automatically generated

Figure 6. The length of *Fucus distichus* zygotes after 5 days of growth in control, low, and high ammonium seawater treatments.

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