Greenland seaweeds for human consumption

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Greenland seaweeds for human consumption

Katharina Johanna Kreissig

PhD thesis

Data sheet

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Summary

Seaweeds, i.e., marine macroalgae, have gained increasing interest as food in the Nordic countries in recent years. Greenland, geopolitically a part of Europe as a self-governing region within the Kingdom of Denmark, currently imports most if its food. Locally harvested and produced seaweed could contribute to a more sustainable food landscape, and open new possibilities for export. Seaweed is a part of the traditional Greenland Inuit diet, though not widely consumed any more. Furthermore, knowledge on the nutritional composition and possible harmful compounds of Greenlandic seaweeds is scarce.

The overall aim of this PhD study was to characterise seaweed species from Greenland regarding their potential use as food items. The aim was therefore to determine the nutritional composition, contaminants, and anti-nutritional factors. Furthermore, the influence of anthropogenic microbial and chemical contamination was investigated. The influence of processing in the form of washing and blanching on the shelf-life of fresh seaweed was also studied. Finally, this project aimed to quantify the impact of increased local seaweed harvesting and culture in Greenland within the framework of the United Nations Sustainable Development Goals (SDGs).

The nine brown and one red seaweed species investigated had different nutritional profiles considering elemental composition (As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Se and Zn), iodine, proteins, fatty acids, amino acids, antioxidants. However, they shared some similarities within groups: the fucoids (*Ascophyllum nodosum*, *Fucus distichus* and *Fucus vesiculosus*) and the kelps (*Hedophyllum nigripes*, *Laminaria solidungula*, *Saccharina latissima* and *Saccharina longicruris*), except for the kelp *Agarum clathratum*, which had a vastly different profile from the other kelps. The red seaweed *Palmaria palmata* also showed a different profile compared to the brown seaweeds investigated.

High iodine content was identified as an issue in all brown seaweeds, but none of the common contaminants typically associated with seaweed (arsenic, cadmium, lead, and mercury) were at concerning levels. However, due to high potassium concentrations, patients on low potassium diets would obtain a significant part of their recommended daily intake from the consumption of *H. nigripes*, *L. solidungula* or *S. longicruris*. Kainic acid was detected in *P. palmata* but was not evaluated to be a hazard.

Anthropogenic contamination was both evident in elemental profiles as well as through microbial contamination. Therefore, it is recommended to avoid harvesting close to, and downstream from, wastewater discharge into the sea.

A shelf-life study of Danish *S. latissima* washed or blanched in either potable or seawater suggested a maximum refrigerated shelf-life of 7 days, stored at 3 ℃. Bacterial spoilage was driven by *Pseudomonas* spp. and *Shewanella* spp. Blanching successfully increased some pos-

itive odour attributes and changed the colour of the seaweed from brown to green, making it an interesting tool for culinary experiments, and product development. This study serves as an important contribution to the understanding of food quality and storage life in general and should be repeated with Greenlandic seaweed to validate the findings.

An increased seaweed harvest and cultivation in Greenland would most positively impact SDGs 8 (Decent work and economic growth), 12 (Responsible consumption) and 14 (Life below water), and most negatively SDG 13 (Climate action). Reducing energy consumption and shifting to renewable energy sources for harvesting, culture and processing could mitigate some of this negative impact.

In summary, the overall evaluation of the chemical, nutritional and microbial assessment shows that all ten species investigated are suitable for human consumption, when harvested away from contamination sources. It is therefore recommended to promote seaweed consumption through the public health program Inuuneritta III, and the SDG agenda of Greenland. The results from this project can be directly used to inform consumers about the nutritional properties of Greenlandic seaweeds, and as a basis for future research of further interesting components.

Sammenfatning

Tang, dvs. marine makroalger, har gennem de seneste år fået stigende opmærksomhed som mad i de nordiske lande. Grønland, geopolitisk en del af Europa som en selvstyrende område inden for Kongeriget Danmark, importerer i øjeblikket de fleste fødevarer. Lokalt høstet og produceret tang kunne bidrage til et mere bæredygtigt madlandskab og åbne nye muligheder for eksport. Tang er en del af den traditionelle grønlandske inuit kost, men forbruget er ikke længere udbredt. Derudover mangler der viden om den ernæringsmæssige sammensætning og mulige indhold af skadelige stoffer i den grønlandske tang.

Det overordnede formål med denne ph.d. studie var at karakterisere tangarter fra Grønland med hensyn til deres potentielle anvendelse som fødevarer. Målet var derfor at bestemme den ernæringsmæssige sammensætning, samt indhold af forurenende stoffer og anti-ernæringsmæssige faktorer. Endvidere blev indflydelsen af menneskeskabt mikrobiel og kemisk forurening undersøgt. Indflydelsen af forarbejdning i form af vask og blanchering på holdbarheden af frisk tang blev også undersøgt. Endelig havde dette projekt til formål at kvantificere effekten af øget lokal høstning og dyrkning af tang i Grønland inden for rammerne af de Forenede Nationers 17 verdensmål for bæredygtig udvikling.

De undersøgte ni brune og en rød tangarter havde forskellige ernæringsprofiler i forhold til elementær sammensætning (As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Se og Zn), jod, proteiner, fedtsyrer, aminosyrer og antioxidanter. Ligheder kunne observeres inden for grupper: fukoider (*Ascophyllum nodosum*, *Fucus distichus* og *Fucus vesiculosus*) og store bladtangarter (*Hedophyllum nigripes*, *Laminaria solidungula*, *Saccharina latissima* og *Saccharina longicruris*), bortset fra den store bladtang *Agarum clathratum*, som havde en meget forskellig profil fra de andre store bladtangarter. Den røde tangart *Palmaria palmata* viste også en anden profil sammenlignet med de undersøgte brune tangarter.

Et højt jodindhold blev identificeret som et problem i alle brune tangarter, men ingen af de almindelige forurenende stoffer, der typisk er forbundet med tang (arsen, cadmium, bly og kviksølv), var på bekymrende niveauer. På grund af høje kaliumkoncentrationer ville patienter på en lav kalium diæt imidlertid få en betydelig del af deres anbefalede daglige indtag dækket ved at spise *H. nigripes*, *L. solidungula* eller *S. longicruris*. Kaininsyre blev påvist i *P. palmata*, men blev på grund af et lavt relativt indhold ikke vurderet til at være en fare.

Menneskeskabt kontaminering af tangens omkringliggende miljø resulterede i tydelig ændringer i tangens elementære og mikrobiologisk profiler med risiko for forekomst af sygdomsfremkaldende mikroorganismer. Derfor anbefales det at undgå høst tæt på og nedstrøms af spildevandsudledninger til havet.

En holdbarhedsundersøgelse af dansk *S. latissima* vasket eller blancheret i enten drikkevand

eller havvand tydede på en maksimal holdbarhed på 7 dage på køl (3 ℃). Bakteriel fordærv blev drevet af *Pseudomonas* spp. og *Shewanella* spp. Blanchering øgede flere positive lugtattributter og ændrede tangens farve fra brun til grøn, hvilket gør blanchering til et interessant værktøj i kulinariske eksperimenter og produktudvikling. Denne undersøgelse tjener som et vigtigt bidrag til fødevaresikkerhedsfeltet generelt og bør gentages med grønlandsk tang for at validere resultaterne.

En øget høstning og dyrkning af tang i Grønland ville have mest positivt indflydelse på verdensmål 8 (Anstændige jobs og økonomisk vækst), 12 (Ansvarligt forbrug og produktion) og 14 (Livet i havet) og mest negativ indflydelse på verdensmål 13 (Klimaindsats). At reducere energiforbruget og skifte til vedvarende energikilder til høst-, dyrknings- og forarbejdningsaktiviteter kan mindske noget af denne negative effekt.

Sammenfattende viser den samlede vurdering af de kemiske, ernæringsmæssige og mikrobielle analyser, at alle ti undersøgte arter er egnede til konsum, når de høstes på afstand fra forureningskilder. Det anbefales derfor at fremme tangforbruget gennem folkesundhedsprogrammet Inuuneritta III og dagsordenen for de 17 verdensmål i Grønland. Resultaterne fra dette projekt kan bruges direkte til at informere forbrugerne om ernæringsmæssige egenskaber af grønlandsk tang og som grundlag for fremtidig forskning i yderligere interessante komponenter.

Publications

Characterisation and chemometric evaluation of 17 elements in ten seaweed species from Greenland

Katharina J. Kreissig, Lisbeth Truelstrup Hansen, Pernille Erland Jensen, Susse Wegeberg, Ole Geertz-Hansen, Jens J. Sloth
https://doi.org/10.1371/journal.pone.0243672
Dataset: Elemental analysis results of Greenland seaweeds. Technical University of Denmark. https://doi.org/10.11583/DTU.13251575.v1

Nutritional composition of ten seaweeds from Greenland

Katharina J. Kreissig, Lisbeth Truelstrup Hansen, Susse Wegeberg, Ole Geertz-Hansen, Charlotte Jacobsen and Susan Løvstadt Holdt
Draft intended for Current Research in Food Science

Microbiota of bladderwrack harvested in and outside areas impacted by human sewage in Greenland

Katharina J. Kreissig, Jonas Steenholdt Sørensen, Lisbeth Truelstrup Hansen, Pernille Erland Jensen
Draft intended for Marine Pollution Bulletin

Conference contributions

Laptop presentations

"Seaweeds – a sustainable food resource from Greenland" by KJ Kreissig, LT Hansen, PE Jensen, JJ Sloth, Sustain 2018, Kongens Lyngby, Denmark, November 2018

Oral presentations

"Grønlandsk tang som fødevare - et PhD projekt" by KJ Kreissig, Seaweed workshop during Greenland Science Week 2019, Nuuk, Greenland, December 2019

Posters

"Faecal bacteria on seaweeds in Greenland" by KJ Kreissig, LT Hansen, PE Jensen, S Wegeberg, O Geertz-Hansen, 7th Nordic Seaweed Conference, Grenaa, Denmark, October 2017

"Greenland seaweeds for human consumption" by KJ Kreissig, LT Hansen, Arktisk forskning og teknologi konference 2017, Copenhagen, Denmark November 2017

"Seaweeds as a new food resource from Greenland" by KJ Kreissig, LT Hansen, Sustain 2017, Kongens Lyngby, Denmark, December 2017

"Characterization of 17 elements in ten edible seaweed species from Greenland" by KJ Kreissig, SL Holdt, BK Herbst, PE Jensen, LT Hansen, JJ Sloth, 23rd International Seaweed Symposium, Jeju, South Korea, May 2018

"Should we harvest the seaweed that grows right in town?" by KJ Kreissig, PE Jensen, LT Hansen, Greenland Science Week 2019, Nuuk, Greenland, December 2019

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1 Introduction

1.1 Seaweed bioeconomy

Seaweeds have a wide range of usage, from large volume, low value applications such as in environmental remediation, to small volume, high value applications in medicine, as illustrated in figure 1.1. This PhD project focuses on the application of seaweeds as food, since there is an increasing demand for food. The global population is predicted to increase to 9.7 billion by 2050 [229]. Reducing food waste will certainly contribute to bridging the gap between supply and demand, since an estimated 33 to 50 % of the world food production is not consumed [216]. However, increased food production will also be necessary [84]. Here, seaweed production offers a solution that circumvents some of the major constraints of land-based agriculture, namely the need for land, freshwater, pest-control (pesticides insecticides and fungicides) and fertilisers [90].

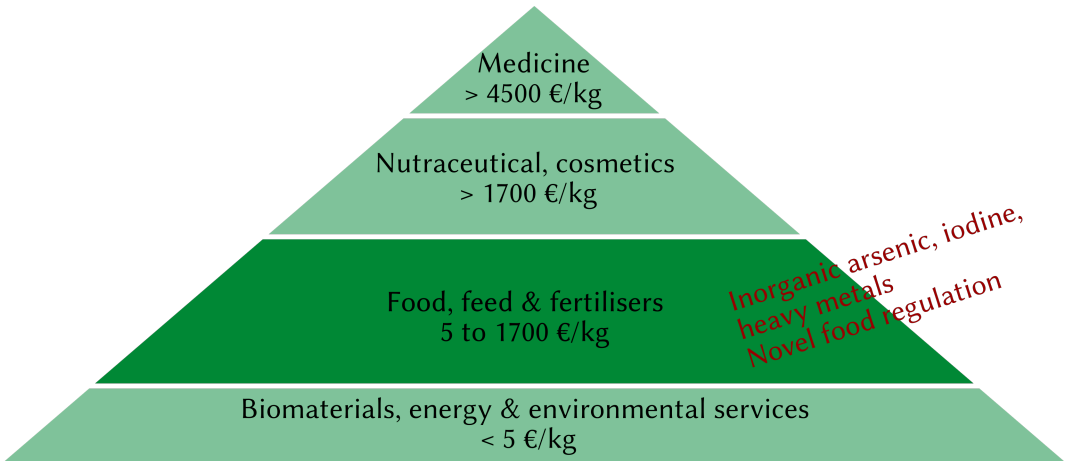

Figure 1.1: Value pyramid for seaweed applications with some of the major constraints for utilization in the fields of food, feed and fertilisers. This PhD project focuses on the use of seaweeds as food, with implications for the nutraceuticals area. Figure freely adapted from Barbier et al. [13] and Hasselström et al. [90].

The use of seaweeds as food currently faces several constraints in the forms of potentially undesirable concentrations of certain substances (such as inorganic arsenic, iodine and several heavy metals) and in the European Union (EU), the Novel food regulation (see text box below for the definition of novel food) [13].

> 'Novel food' means any food that was not used for human consumption to a significant degree within the Union before 15 May 1997, [...] and that falls under at least one of the following categories: [...]
> (ii) food consisting of, isolated from or produced from microorganisms, fungi or algae. [...] European Council Regulations No. 2015/2283 [74]

Foods, and seaweeds, that are not included in the EU Novel food catalogue[1] as non-novel need to be certified before they can be put onto the market [74].

In Europe, the seaweed sector has the potential for considerable sustainable development, and to contribute to the EU Bioeconomy Strategy [8].

The five goals of the European bioeconomy strategy European Commission [64]:

- ensure food and nutrition security
- manage natural resources sustainably
- reduce dependence on non-renewable, unsustainable resources
- limit and adapt to climate change
- strengthen European competitiveness and create jobs

1.2 Seaweed and Greenland

Greenland, while geographically part of Northern America, is geopolitically a part of Europe as a self-governing region within the Kingdom of Denmark [26]. Fisheries is the main economic income source of Greenland with a turnover of DKK 6.5 billion in 2019 [26] and the second biggest employer. Decreased sea ice thickness and coverage, combined with increasing sea surface temperatures, have led to changing patterns of species abundance and diversity [75], which may change the species available for fisheries. Thus, climate change is both a challenge and an opportunity, rendering the exploitation of alternative resources such as seaweeds attractive. Seaweed could provide an environmentally friendly income source with manageable investment requirements, making use of already existing infrastructure. Furthermore, the current diet pattern in Greenland is leaning heavily towards a Danish diet [92], with most food imported. Local seaweed could provide a more sustainable, and potentially healthier, alternative to some of the imported foods [113].

With 44 087 km coastline [26], low water pollution, and a diverse native seaweed population, Greenland has many of the prerequisites to make seaweed cultivation a viable business [235]. Currently, seaweed exploitation in Greenland is hindered by a general lack of knowledge on

[1] Available online at https://ec.europa.eu/food/safety/novel_food/catalogue_en

seaweeds, and especially their nutritional composition. Locals are also worried about pollution sources. Seaweed cultivation is at the very beginning (see also chapter 4, page 21), and apart from any issues related to the farming process, the correct processing of harvested seaweed, such as washing, is one of the challenges.

1.3 Objectives and hypotheses of the thesis

The overall objective of this PhD study was to characterise seaweeds from Greenland with regard to their potential use as a food item.

The research was structured into four specific objectives:

- Determine the nutritional composition, including contaminants and anti-nutritional factors
- Investigate the influence of anthropogenic contamination
- Examine the influence of processing in the form of washing and blanching on shelf-life of fresh seaweed
- of increased local seaweed harvesting and culture on Greenland within the framework of the United Nations Sustainable Development Goals (SDGs)

The five main hypotheses of the work were:

1. The content of elements in Greenland seaweed species depends on species, thallus part and geographic origin.
2. Seaweed harvested close to contamination sources are unsuitable for human consumption due to the presence of foodborne pathogens.
3. Processing in the form of washing in freshwater has a detrimental effect on the shelf-life of fresh seaweed.
4. Processing in the form of blanching can extend the shelf-life of fresh seaweed.
5. The increased harvesting, production and consumption of local seaweed in Greenland has a positive impact measured through the SDGs.

1.4 Structure of the thesis

Introduction (the present chapter)

Experimental approach – Explains the choice of sampling locations, species, and analytical methods and presents an overview of the workflow (page 7)

Background information

- Seaweed biology – A brief primer on seaweed ecology and the seaweed species investigated in this project (page 11)
- Consumption and production of seaweed in Greenland – A brief primer on historical and current use of seaweed in Greenland (page 21)

Results

- The composition of raw seaweed
  - Micronutrients and iodine - Selected micronutrients: iodine and 16 other elements (page 25)
  - Lipids, amino acids, bioactive components, dry matter and ash - Selected macronutrients (page 49)
  - The influence of anthropogenic contamination on the microbiota of seaweed (page 73)
  - Kainic acid in *P. palmata* (page 93)
- The influence of processing
  - Shelf-life of washed or blanched Danish sugar kelp (page 97)
- The potential impact of the research project
  - Impact on Sustainable Development Goals (page 115)

Conclusions and future research perspectives - overarching summary and concluding remarks (page 124) and perspectives for future research (page 127)

References (page 131)

Appendices (page 153)

2 Experimental approach

2.1 Choice of sampling locations

The project aimed to sample seaweed from as many geographical locations in Greenland as possible, see figure 2.1 for an overview. Sampling was performed by several members of the project team, who were able to obtain samples in conjunction with this project, as well as other projects and teaching activities. Intensive sampling of a larger community, Sisimiut, where DTU has a satellite campus, and a nearby small community, Sarfannguit, were also carried out over two harvest seasons (2017 and 2018). The investigation of anthropogenic influence of

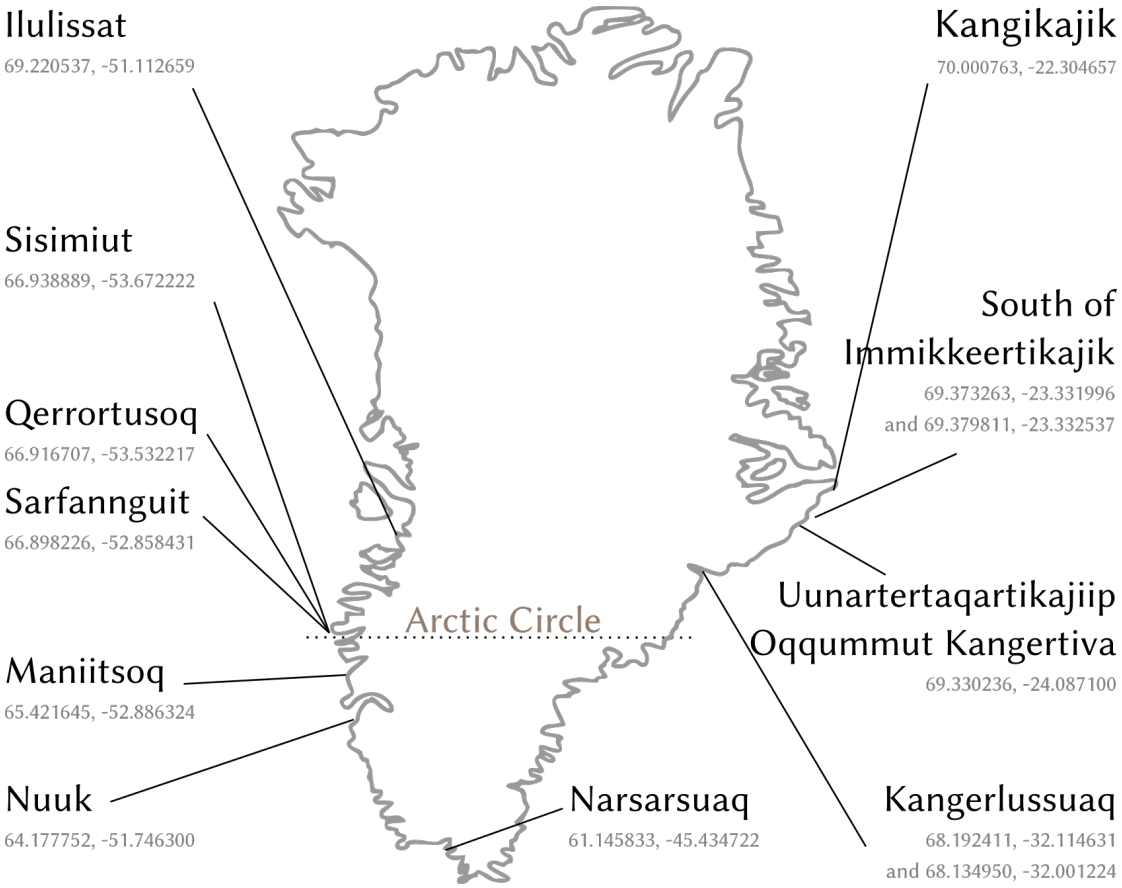

Figure 2.1: Sampling locations in Greenland with coordinates in decimal degrees (latitude, longitude). For Sarfannguit, coordinates are given for one central location (fish factory), the dump (66.897536, -52.874138) and school coordinates (66.896311, -52.857659) were omitted from the figure due to stylistic choice.

contamination on seaweed was carried out in the town of Sisimiut and the nearby settlement of Sarfannguit to minimise the influence of geographical differences on the results, and to allow

for sampling at both locations within a very short timeframe. Furthermore, Sisimiut is the second largest town in Greenland with over five thousand inhabitants, while Sarfannguit can be considered a representative of a smaller settlement with around one hundred inhabitants [26]. Also, DTU Civil Engineering is carrying out different research activities in Sarfannguit, which made revisiting in 2018 for further sampling possible.

The sampling locations and seaweeds sampled in remote parts of Eastern Greenland were part of an independent survey carried out by co-supervisors Susse Wegeberg and Ole Geertz-Hansen in 2017.

2.2 Choice of seaweed species

The seaweed species investigated were selected because they are already used as foods in Greenland or other Nordic countries (*Alaria esculenta*, *Ascophyllum nodosum*, *Fucus vesiculosus*, *Palmaria palmata*, *Saccharina latissima*, *Saccharina longicruris*), have the potential to become an interesting food item (*Fucus distichus*, *Hedophyllum nigripes*, *Laminaria solidungula*), or are potentially rich in bioactive components (*Agarum clathratum*). Of these, *A. clathratum*, *F. distichus*, *H. nigripes* and *L. solidungula* fall under the category of novel foods and would therefore require certification for the EU market.

More information on the individual species can be found in section 3.5, page 14.

2.3 Choice of working with Danish *Saccharina latissima* for shelf-life study

Due to the travel restrictions caused by the coronavirus disease (COVID-19) outbreak, the planned 2020 field work in Greenland was replaced by a study on the shelf-life of *Saccharina latissima* cultivated in Danish waters. The results will be valuable for future research on the shelf-life of fresh Greenlandic seaweeds. Moreover, *S. latissima* is currently cultivated on experimental basis in Greenland.

2.4 Choice of analytical methods

Since this project was not concerned with method development, proven and tested methods were used. Standard analytical methods were carried out at the National Food Institute, Kongens Lyngby, Denmark. Sequencing of microbial DNA extracted from seaweed for the study of contamination on seaweed was carried out by Eurofins Institut Jäger GmbH Konstanz, Germany.

2.5 Overview of materials used in the different investigations

Figure 2.2 presents an overview of the overarching workflow. It identifies which materials were used in which studies.

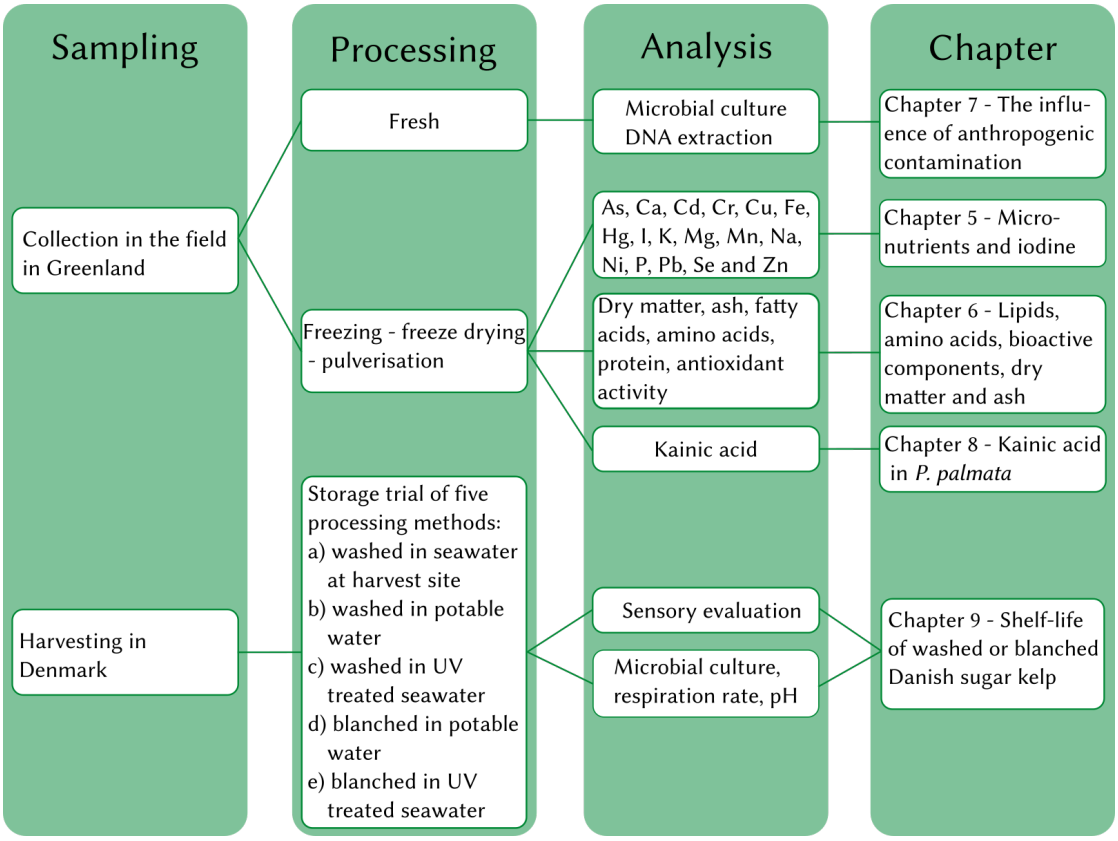

Figure 2.2: Schematic of collected sample materials, processing and results.

3 Seaweed biology

3.1 Habitat

Seaweeds (marine macroalgae) can mainly be found from the littoral zone (also called tidal zone) down into the sublittoral zone (also called subtidal zone) where sunlight eventually becomes a limiting factor [38, 175, 236]. The littoral zone is exposed to air but covered by the tides twice per day, while the sublittoral zone is permanently submerged. For a schematic drawing of the littoral and sublittoral zone with focus on the species investigated in this project, see figure 3.1 (p. 12).

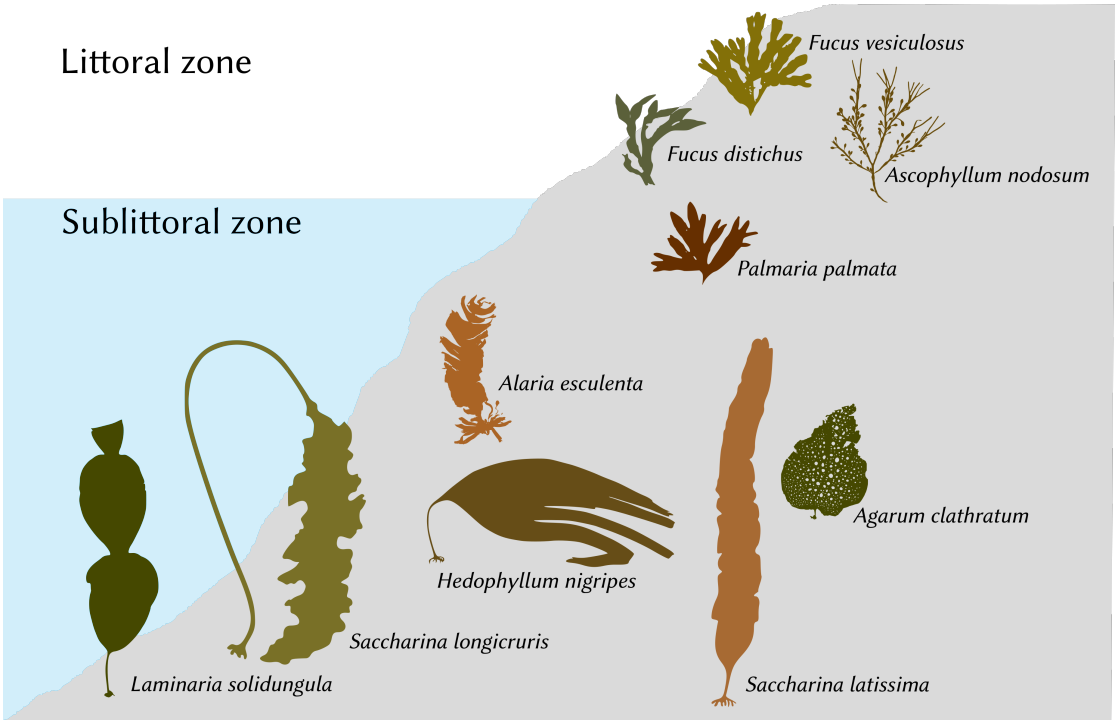

Figure 3.1: Schematic of seaweeds in the littoral (also called tidal) and sublittoral (also called subtidal) zone in Greenland. The relative growth zones of all ten seaweed species investigated in this project are shown. Not to scale.

Seaweeds are important parts of marine ecosystems and may create a belt of vegetation in the littoral zone and underwater forests, consisting of large brown seaweeds (kelp forests) [236]. Seaweeds, especially in the tidal zone and in kelp forests, provide structure for a sheltering habitat, and they may be inhabited by species of invertebrates, such as sea urchins, species of molluscs, crustaceans; and fish [38, 132, 200, 237]. In Greenland, fish associated with kelp forests include the commercially important Atlantic cod *Gadus morhua* (Linnaeus, 1758) as well as sculpins [200]. In Greenland, birds such as the black guillemot *Cepphus grylle* (Linnaeus, 1758) forage the seaweed for these fish and invertebrates [23]. In Norway, Lorentsen, Sjøtun,

and Grémillet [132] demonstrated that kelp harvesting significantly reduced the number of small gadid fish, and greatly increased the number of dives of a marine top predator bird, the great cormorant (*Phalacrocorax carbo*, Linnaeus, 1758).

Seaweeds depend on several abiotic factors for growth: light, nutrients, temperature, salinity as well as the availability of a firm substrate for being attached [175]. There are several other factors, which can have a negative impact on the establishment and growth of seaweeds and affect the species composition at a given site: grazing, wave exposure, suspended sediments that increase the water turbidity, and especially for Greenland, scouring of the upper metres of the shoreline by drifting ice, and by winter ice cover which may reduce light availability [175, 236]. The formation of an ice foot[1] may both protect the seaweeds frozen inside as well as damage them when it breaks before melting completely [236].

3.2 Seaweed morphology

Seaweeds display a wide range of different morphologies. The entire body of a seaweed is called thallus (plural thalli). Seaweeds are usually anchored to stable surfaces by a holdfast. For some species, a stipe connects the holdfast to the leaf-like structure called blade or lamina that forms the uppermost part of the thallus [17]. In other species, gas filled air bladders can be found in the blade, these improve the buoyancy of the seaweed, keeping it upright in the water column to improve its access to light [17]. In figure 3.2 (p. 14), representatives of each of the three groups investigated in this project are shown: fucoids, kelps and red seaweed (with leaf-shaped thalli).

3.3 Physiological functions of seaweed constituents

The biochemistry of seaweeds can broadly be divided into three types: structural constituents, such as carbohydrates, defence mechanisms, such as polyphenols [17] and iodide [118], and thirdly, non-functional constituents, which are passively taken up, such as some of the heavier divalent metals [157] (see also chapter 6, page 49).

3.4 Seaweed life histories

Seaweeds differ from higher plants in that they are spore plants and have mobile stages as part of their life histories, which also include different generations [10]. The individual life histories of seaweeds are complex and differ significantly between species [10]. These different life histories may have specific environmental condition requirements, e.g., day length, temperature, and which need to be known for successful cultivation of a species in a specific location

[1] Definition of ice foot from Merriam Webster: a wall or belt of ice frozen to the shore in Arctic regions having a base at or below the low-water mark and formed as a result of the rise and fall of the tides, freezing spray, or stranded ice https://www.merriam-webster.com/dictionary/ice%20foot

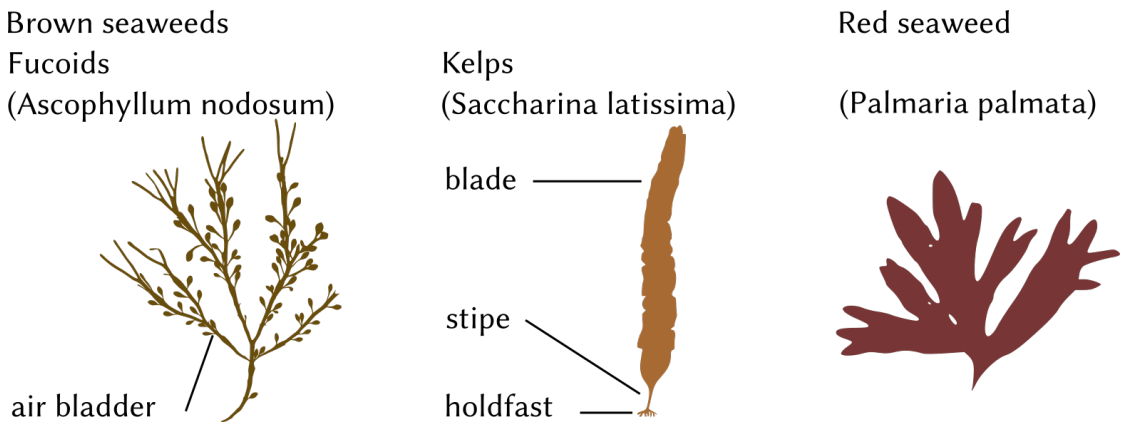

Figure 3.2: Schematic of brown seaweeds (fucoids, kelps) and the red seaweed species which were investigated in this project. Not all fucoids (refer to section 3.5.1, page 15) have air bladders, while some kelps have midribs. The red seaweed *Palmaria palmata* (section 3.5.3, page 19) has a leaf-shaped thallus, which is, however, not the case for all red seaweeds.

[10]. Since the focus of this project is on the nutritional contents and suitability for human consumption of Greenland seaweeds, for the detailed descriptions of three very different life histories, please refer to Tarakhovskaya et al. [223] for *Fucus vesiculosus* (for morphological and habitat description of the species, see section 3.5.1, page 16), Redmond et al. [184] for *Saccharina latissima* (see section 3.5.2, page 18)), and Grote [86] for *Palmaria palmata* (see section 3.5.3, page 19).

3.5 Greenland seaweeds

Seaweeds can be divided into three main groups, which correspond to their colour: Chlorophyta (green), Phaeophyta (brown) and Rhodophyta (red) [175]. In Greenland, about 200 distinct species of seaweeds can be found [175]. Of these, around 80 are brown algae, 50 red and 50 green algae [175]. Brown algae dominate both the tidal zone as well as the sublittoral zone with very high biomasses and a high productivity [175, 236, 237]. The species richness is gradually decreasing from South to North along the coast of Greenland [175]. This may in part be due to less favourable growth conditions further north, especially with relation to the extreme seasonality regarding length of the day and low temperatures [236]. However, high standing biomasses can still be found far north, with species adapted to the environmental conditions by for example being able to initiate growth under suboptimal light conditions [236]. The East and West coast of Greenland have differing seaweed distributions, with some species documented along almost the entire West coast, but only partway up the east coast [175, 236].

In the following sections, the ten species investigated in this PhD project are described

briefly, grouped into brown seaweeds (fucoids, kelps) and red seaweed, and then further sorted alphabetically by their species names. Their species names are given in accordance with Pedersen [175] and AlgaeBase[2], Danish and Greenlandic in accordance with Asimi[3], as well as Andersen et al. [4]. The distribution along both the west and east coast described here is collected from literature, and non-exhaustive. Especially the distribution along the east coast has only been described in limited areas of the entire coastline.

3.5.1 Fucoids

Ascophyllum nodosum, knotted wrack

Ascophyllum nodosum (Linnaeus) Le Jolis 1863

EN: knotted wrack DA: buletang KL: sapangaasat
Thallus type: very large branched thalli
Distribution: Greenlandic west coast 60° N to 73° N [205, 232, 233, 234]; Greenlandic east coast 65° N [236]

Ascophyllum nodosum can be found in the tidal zone [237] and the uppermost part of the sublittoral zone [175]. It is typically found along a coastline that is protected against wave exposure and ice scouring. *Ascophyllum nodosum* is easily recognisable by the band-like thallus structure interspersed with relatively big and singularly placed air bladders for buoyancy. The entire thallus can be up to 100 cm long. In Greenland, it forms its reproductive structures, which resemble small yellow gooseberries, during the summer [175].

Fucus distichus, rockweed

Fucus distichus Linnaeus 1767

EN: rockweed DA: smal klørtang KL:
Thallus type: very large branched thalli
Distribution: Greenlandic west coast 62° N to 67° N [234]; Greenlandic east coast 68° N and 71° N to 73° [236]

Fucus distichus typically forms the lower part of the fucoid belt in the tidal zone (S. Wegeberg, personal communication, February 22nd, 2021). It is also one of the dominant species in seaweed meadows in East Greenland, between around 71° N and 73° N [236]. These meadows were typically observed around 10 m depth, but with a range from 5 to 20 metres. The flattened thallus can be up to 100 cm long and has a midrib. *Fucus distichus* from the Atlantic coast of Canada display a range of morphologies [117] and may be difficult to distinguish from *Fucus*

[2] AlgaeBase is a global algal database of taxonomic, nomenclatural, and distributional information, https://www.algaebase.org/
[3] Asimi is a web based teaching resource on Greenland's nature, http://asimi.gl/

vesiculosus without bladders in the field. However, in contrast to *F. distichus*, *F. vesiculosus* has reproductive structures that produce either sperm or eggs [175] (S. Wegeberg, personal communication, February 22nd, 2021).

Fucus vesiculosus, bladderwrack

Fucus vesiculosus Linnaeus 1753

EN: bladderwrack DA: blæretang KL: equutit
Thallus type: very large branched thalli
Distribution: Greenlandic west coast 60° N to 71° N [232, 233, 234]; Greenlandic east coast 65° N and 70° N to 76° N [236]

Fucus vesiculosus is a very common macroalgae of the tidal zone [175, 237]. The branched thallus, which can be 80 to 100 cm long, has a midrib, and is characterised by pairwise air bladders in the more blade shaped parts. In very small or stressed specimens in the uppermost part of the tidal zone these air bladders may be absent from the thallus [175].

3.5.2 Kelps

Agarum clathratum, sieve kelp

Agarum clathratum Dumortier 1822

EN: sieve kelp DA: hultang KL: putoortut; kipilatsad [4]
Thallus type: metres-long thalli
Distribution: Greenlandic west coast 60° N to 77° N [111, 205, 232, 233, 234]; Greenlandic east coast 66° N to 68° N [236]

Agarum clathratum is one of the most common types of laminaria seaweeds on the Greenland west coast [175]. Wegeberg [235] found that *A. clathratum* made up about 29% (1 kg m^{-2}) of biomass in Southwest Greenland, at an average age of two to three years, with individuals up to seven years old. This species has a branched holdfast, with a stipe that can be up to 30 cm long. The stipe continues as a midrib through the lamina, which has small, characteristic holes [111, 175]. The lamina can grow to a width of 50 cm and a length of 90 cm. *Agarum clathratum* can be found from the uppermost part of the sublittoral zone down to depths of about 40 metres [175]. However, a recent study of offshore kelp forests in the Disko Bay region (67 °N to 70 °N, west coast of Greenland) found *A. clathratum* at more than 61 metres of depth, which is also an indication of very clear water [111]. Wegeberg [235] found a negative correlation between fetch[4] and the total biomass of *A. clathratum*.

[4] Fetch is the open distance facing the coast, and in which wind can take up speed. Wind fetch is hence used as proxy for wind exposure and resulting strength of waves.

Alaria esculenta, winged kelp

Alaria esculenta (Linnaeus) Greville 1830

EN: winged kelp DA: vingetang KL: sulluitsoq
Thallus type: metres-long thalli
Distribution: Greenlandic west coast 60° N to 70° N [232, 233]; Greenlandic east coast 66° N to 76° N [236]

Alaria esculenta is often found at wave exposed locations. Hence, Wegeberg [235] found a positive correlation between wind fetch and the total biomass of *A. esculenta*. *Alaria esculenta* made up about 30% of biomass (1 kg m^{-2}) in a study in Southwest Greenland, with an average age of 3.3 years, with individuals up to eight years old [235]. This species is characterised by a stipe that continues throughout the blade as a midrib. Between the holdfast and the stipe, small blades protrude, the sporophylls, which carry the reproductive structures. *Alaria esculenta* typically grows to be between 2 to 3 metres long but may be even longer. In East Greenland (between 70 °N to 76 °N), *A. esculenta* may dominate the kelp forests until depths of about 25 meters, together with *S. latissima* and, especially further North, *Laminaria solidungula* [236].

Hedophyllum nigripes, blackfoot kelp

Hedophyllum nigripes (J.Agardh) Starko & S.C.Lindstrom & Martone 2019

EN: blackfoot kelp DA: sortfods bladtang KL: qernaluk
Thallus type: metres-long thalli
Distribution: Greenlandic west coast 62° N to 71° N [232, 234]; Greenlandic west and east coast 70° N [236]

Hedophyllum nigripes can be found from the uppermost part of the sublittoral zone and in low lying tidal pools [175]. In Southwest Greenland, *H. nigripes* contributed on average 15% (0.5 kg m^{-2}) of kelp biomass in a study by Wegeberg [235], with an average specimen age of two to three years. Wegeberg [235] found a positive correlation between fetch and the total biomass of *H. nigripes*. The stipe supports a blade of up to 80 cm length and 30 cm width (asimi.gl). The stipe and the lower part of the blade turn blackish, when dried up.

Laminaria solidungula, Arctic suction-cup kelp

Laminaria solidungula J. Agardh 1868

EN: Arctic suction-cup kelp DA: skive bladtang KL: qanallarnaq
Thallus type: metres-long thalli
Distribution: Greenlandic west coast 60° N to 71° N [232, 233, 234]; Greenlandic east coast 71° N to 76° N [236]

Laminaria solidungula is attached to the substrate with an adhesive disc and can be found at depths below 10 metres. The blade is segmented by constrictions, which denote the yearly growth [236], with a total length of 50 to 110 cm [18] or even longer. *Laminaria solidungula* is not often found at the west coast, and more commonly found on the East Coast of Greenland (O. Geertz-Hansen, S. Wegeberg, personal communication, February 9th, 2021): between 70 °N to 76 °N, and especially in the northernmost part of this range, *L. solidungula* together with *A. esculenta*, were the major species of the kelp forests [236]. Wegeberg [235] found *L. solidungula* at the Southwest coast of Greenland, but at less than 1% of biomass (reported together with another kelp species).

Saccharina latissima, sugar kelp

Saccharina latissima (Linnaeus) C.E. Lane & C. Mayes Druel & G.W. Saunders 2006

EN: sugar kelp DA: sukkertang KL: uisuk
Thallus type: metres-long thalli
Distribution: Greenlandic west coast 60° N to 76° N [111, 232, 233, 234]; Greenlandic east coast 66° N to 76° N [236]

Saccharina latissima is a very common seaweed, it grows from 2 to 30 m depth. However, a recent study of west coast offshore kelp forests found evidence of *S. latissima* and *S. longicruris* down to depths of more than 51 metres [111]. Wegeberg [235] found that *S. latissima* made up about 25% (0.9 kg m^{-2}) of biomass in Southwest Greenland, with an average age of two to three years. This species has a solid stipe and a blade with ruffled sides of up to 3 metres in length [175]. *Saccharina latissima* is usually found in semi-exposed locations, and as such Wegeberg [235] found no correlation between wind fetch and the total biomass of *S. latissima.* In East Greenland, *S. latissima* may dominate the kelp forests (between 70 °N to 76 °N), together with *A. esculenta* and *L. solidungula*, until depths of about 25 meters [236].

Saccharina longicruris, Northern rhizome kelp

Saccharina longicruris (Bachelot de la Pylaie) Kuntze 1891

EN: Northern rhizome kelp DA: langstilket bladtang KL: qeqquaq
Thallus type: metres-long thalli
Distribution: Greenlandic west coast 63° N to 78° N [111, 232, 234]

Saccharina longicruris has a long, hollow stipe (which distinguishes it from *S. latissima*) of up to 7 metres in length, and a blade width of up to 60 cm. The total length of *S. longicruris* can be up to 10 metres, and it grows at depths of 4 to 12 metres (asimi.gl). However, a recent study of west coast offshore kelp forest found evidence of *S. longicruris* down to depths of more than 51 metres [111].

3.5.3 Red seaweeds

Palmaria palmata, dulse

***Palmaria palmata* (Linnaeus) Weber & Mohr 1805**

EN: dulse DA: søl KL: aappilattut
Thallus type: leaf-shaped thalli
Distribution: Greenlandic west coast 63° N to 78° N [232, 234]

Palmaria palmata is a very common species in the tidal zone and the upper part of the sublittoral zone in not too exposed areas [175]. The leaf-shaped thallus, which can be 5 to 46 cm long [105, 192], is attached to the substrate with an adhesive disc. New blades are formed along the edge of the first formed blade [175].

4 Consumption and production of seaweed in Greenland

Seaweeds have been a part of the traditional diet in Greenland. In early surveys made by Europeans, seaweeds were often described as a food item in times of scarcity [6] and during winter, as a source of vitamin C (Høygaard in [47, 147]). De Bonneval and Robert-Lamblin [47] reported that in the Amassalik area of East Greenland, algae were generally rinsed in freshwater before being eaten raw, or sometimes blanched in the cooking water from preparing seal. Mouritsen and Mouritsen [147] describe an "enduring" tradition in Tasiilaq (East Greenland) of local people going on foraging expeditions for snails and mussels, which are cooked and eaten together with raw sugar kelp (*Saccharina latissima*), harvested at low tide. In a study by Whitecloud and Grenoble [240], investigating traditional knowledge about Greenland plants, several of the interviewed local experts identified seaweeds with food use. Dulse (*Palmaria palmata*) was named as the "favourite seaweed of most people" by one expert. In contrast, another expert classified *Laminaria* sp. as inedible: "Don't eat it or your hair will fall out". Table 4.1 (page 23) provides an overview over seaweeds, which have a history of food use, both from historical and current studies.

Today, seaweeds are consumed in two main ways in Greenland: firstly, locally harvested seaweed, often as part of a more traditional diet [4] and secondly, as part of Asian cuisine, mainly using imported seaweed salads and nori sheets for sushi. Furthermore, seaweed is used as a food supplement (ground, powdered and filled into vegetable capsules, see figure 4.1a, page 24) by some Greenlanders (personal communication Christina Hardenberg). Users report these seaweed supplements to be effective against gout pain in the hands, and beneficial effects on skin and nails. Efforts to promote local seaweeds as part of a modern diet also exist, for example by Inunnguaq Hegelund, a famous Greenlandic chef [188], or by online recipe collections such as mamarisavut[1].

Locally harvested seaweed is also used in scientific studies: Noahsen and her collaborators used a sushi meal consisting of a halibut maki roll (containing a sheet of imported nori) and a seaweed salad, either a store bought import, or one freshly prepared for the scientific study from fresh locally harvested seaweed [162] in a study on iodine intake and thyroid excretion.

However, commercial production of seaweed through harvesting or culture is very limited. At the start of this PhD project in 2017, there was one local producer of seaweed in Sisimiut, Maki Seaweed, who sold their products locally, see figure 4.1b and 4.1c, page 24. However, due to logistic reasons, this one man company was closed in 2018.

In 2017, Halibut Greenland[2] also expressed interest in the future exploitation of seaweed and seaweed based products [155]. However, they do not currently engage in any activities concerning seaweed (E. Sivertsen, CEO of Halibut Greenland ApS, personal communication, January 4th 2021).

Royal Greenland A/S[3] has been conducting a pilot study into the feasibility of seaweed production since 2018. After a successful harvest of 900 kg in 2020 of both *A. esculenta* and *S.*

[1] Mamarisavut (mamarisavut.gl) is the online recipe collection of the general store Pilersuisoq.

[2] Halibut Greenland ApS is a fishing company owned by local fishermen in Northern Greenland focussing on Greenland halibut and Atlantic cod.

[3] Royal Greenland A/S is a large seafood company owned by the Government of Greenland.

Table 4.1: Seaweeds consumed as food in historical and current studies from Greenland.

Type		Species	Literature source
Brown seaweeds	Fucoids	*Ascophyllum nodosum*	Holm 1884, Rasmussen 1919, Høygaard 1936-1937, survey by Robert-Lamblin 1979 in De Bonneval and Robert-Lamblin [47]; Mouritsen and Mouritsen [147]; Andersen et al. [4]
		Fucus sp.	Høygaard 1936-1937 in De Bonneval and Robert-Lamblin [47]
		Fucus vesiculosus	Holm 1884, Rasmussen 1919, Høygaard 1936-1937, survey by Robert-Lamblin 1979 in De Bonneval and Robert-Lamblin [47]; Mouritsen and Mouritsen [147]
	Kelps	*Alaria esculenta* (*Alaria pylaî**)	Holm 1884, Rasmussen 1919, Høygaard 1936-1937, survey by Robert-Lamblin 1979 in De Bonneval and Robert-Lamblin [47]; Mouritsen and Mouritsen [147]
		Alaria sp.	Whitecloud and Grenoble [240]
		Laminaria sp.	Kruuse 1898-1902 in De Bonneval and Robert-Lamblin [47]; Whitecloud and Grenoble [240]
Red seaweeds		*Chondrus crispus*	Andersen et al. [4]
		Delessaria sp.	Kruuse 1898-1902 in De Bonneval and Robert-Lamblin [47]
		Palmaria palmata (*Rhodymenia palmata**)	Holm 1884, Kruuse 1898-1902, Rasmussen 1919, Høygaard 1936-1937, survey by Robert-Lamblin 1979 in De Bonneval and Robert-Lamblin [47]; Mouritsen and Mouritsen [147]; Whitecloud and Grenoble [240]

* Species name in some of the original publications.

latissima, they expect to harvest three tons raw seaweed in 2021 (personal communication Nikoline Ziemer), and to scale up to 1000 kg within five years [43].

In 2021, a pilot study to test the suitability of different sites for macroalgal cultivation will be set up in South Greenland, together with partners in Norway, Iceland and Scotland [60] (B. W. Kvamme, project leader, personal communication, November 25th 2020).

The government of Greenland acknowledges the potential future commercial exploitation of seaweed in a report on the effects of climate change on fisheries [153]. Seaweed falls under the agricultural act, as "other aquaculture", with guidelines for collection, harvesting, reporting

(a) Dried, ground Northern rhizome kelp (*Saccharina longicruris*) and knotted wrack (*Ascophyllum nodosum*), filled in plant-based capsules. Foto copyright Christina Hardenberg.

(b) Supermarket display of dried seaweed (top shelf) in Sisimiut in 2017.

(c) Dried *P. palmata* as sold in Sisimiut in 2017.

Figure 4.1: Seaweed food supplements and dried seaweed for sale.

and exporting [107, 134]. Since 2015, collection of drifting seaweed and harvesting of attached seaweed - except for own consumption - requires a permit [134], as is the case for cultivation and export. There are currently only a few permit holders (N. M. Lund, Head of Section, Ministry of Fisheries, Hunting & Agriculture, personal communication, November 13th 2020).

5 Micronutrients and iodine

Publication information

Characterisation and chemometric evaluation of 17 elements in ten seaweed species from Greenland

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Dataset: elemental analysis results of Greenland seaweeds. Technical University of Denmark. https://doi.org/10.11583/DTU.13251575.v1

5.1 Abstract

Several Greenland seaweed species have potential as foods or food ingredients, both for local consumption and export. However, knowledge regarding their content of beneficial and deleterious elements on a species specific and geographical basis is lacking. This study investigated the content of 17 elements (As, Ca, Cd, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Ni, P, Pb, Se and Zn) in 77 samples of ten species (*Agarum clathratum*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus distichus*, *Fucus vesiculosus*, *Hedophyllum nigripes*, *Laminaria solidungula*, *Palmaria palmata*, *Saccharina latissima* and *Saccharina longicruris*). Element profiles differed between species but showed similar patterns within the same family. For five species, different thallus parts were investigated separately, and showed different element profiles. A geographic origin comparison of *Fucus* species indicated regional differences. The seaweeds investigated were especially good sources of macrominerals (K > Na > Ca > Mg) and trace minerals, such as Fe. Iodine contents were high, especially in macroalgae of the family Laminariaceae. None of the samples exceeded the EU maximum levels for Cd, Hg or Pb, but some exceeded the stricter French regulations, especially for Cd and I. In conclusion, these ten species are promising food items.

5.2 Introduction

Marine macroalgae, commonly known as seaweeds, are increasingly becoming popular as food items in the Nordic countries [149], as well as in Greenland [116, 188], where they have been a part of the traditional Inuit diet [4, 47]. Moreover, seaweeds have been identified as a sustainable income source in the remote and sparsely populated areas of the Northern Periphery and Arctic region of Northern Europe and Greenland – a region with a low population density and pristine waters [165].

Having detailed insight in the nutritional composition of macronutrients (lipids, carbohydrates, proteins, etc.) and minor components, including essential and non-essential elements,

is important for both currently consumed seaweed species and species of interest for future human consumption [74].

Seaweeds have a highly variable nutritional composition [98, 136] but are generally good sources of minerals and iodine [98, 148]. However, in some cases they are also known to contain undesirably high concentrations of certain chemical elements, which have been identified as hazardous: As, Cd, Hg, I and Pb [11, 52, 135, 194]. This is attributed to the accumulation of cations from the seawater through their association with biopolymers [46] or, in the case of iodine in some species, its function as an antioxidant [119, 247].

The contents of elements of concern (such as As, Cd, Hg, I, Ni, Pb) need to be mapped for the individual seaweed species. Currently, there is limited European legislation on maximum levels allowed in seaweeds, with stricter regulations on a national level found only in France. Meanwhile, to assess the dietary exposure of the population through the consumption of seaweeds, the EU is collecting information on the occurrence of As, Cd, Hg, I and Pb in a range of seaweeds and products based on seaweeds during the period from 2018 to 2020 [65].

While the nutritional composition of Nordic seaweeds has been studied intensely in recent years, and is increasingly well described [98, 136], there is a distinct lack of knowledge on the contents of Greenland seaweeds.

To address the lack of knowledge about the nutritional profile of Greenland seaweeds, the present study focused on ten seaweed species of interest, harvested in Greenland. The species chosen are either currently consumed in Greenland or Nordic countries (*Alaria esculenta* (Linnaeus) Greville 1830, *Ascophyllum nodosum* (Linnaeus) Le Jolis 1863, *Fucus vesiculosus* Linnaeus 1753, *Palmaria palmata* (Linnaeus) Weber & Mohr 1805, *Saccharina latissima* (Linnaeus) C.E. Lane & C. Mayes Druel & G.W. Saunders 2006, *Saccharina longicruris* (Bachelot de la Pylaie) Kuntze 1891), might conceivably be consumed (*Fucus distichus* Linnaeus 1767, *Hedophyllum nigripes* (J.Agardh) Starko & S.C. Lindstrom & Martone 2019, *Laminaria solidungula* J. Agardh 1868), or are potentially rich in bioactive components (*Agarum clathratum* Dumortier 1822). Since for some of the species, different parts of the thallus, the "body" of the macroalga, can constitute different products, they were analysed separately.

We hypothesise that the content of elements in Greenland seaweed species depends on species, thallus part and geographic origin. To study these hypotheses and to assess the suitability of these species as food items, the objectives of this study were to: (1) investigate the contents of beneficial and toxic elements in a range of Greenland seaweed species, (2) compare the element concentrations between different species, (3) investigate differences between thallus parts for selected species, (4) investigate the influence of geographic origin for *Fucus* spp., and (5) assess the benefits and limitations of the studied seaweeds as food items, through their contribution to recommended dietary intakes (RDIs), respectively the toxicological guideline values.

The findings from this study will be valuable for those currently collecting, farming, processing, marketing and consuming seaweeds in Greenland as well as the future development of the local seaweed sector.

5.3 Materials and methods

5.3.1 Samples and sampling locations

A total of 77 samples belonging to ten seaweed species were collected in the intertidal or upper subtidal zone between June and September in 2017 and 2018 at low tide conditions along the shore or by divers in West, South and East Greenland, see Fig 5.1. Table 5.1 provides an overview of the number of species per location. The harvest sites were chosen to represent different areas in Greenland. The species and number of samples were as following: *Agarum clathratum* (3), *Alaria esculenta* (9), *Ascophyllum nodosum* (7), *Fucus distichus* (8), *Fucus* spp. (7, specimens that were too small to be distinguished as either *F. distichus* or *F. vesiculosus*), *Fucus vesiculosus* (15), *Hedophyllum nigripes* (5), *Laminaria solidungula* (6), *Palmaria palmata* (2), *Saccharina latissima* (10) and *Saccharina longicruris* (3).

Table 5.1: Summary of Greenland seaweed samples included in the study. Coordinates in decimal degrees.

Species	n	Location	Latitude	Longitude
Agarum clathratum	2	Kangerlussuaq	68.134950	-32.001224
Agarum clathratum	1	Maniitsoq	65.421645	-52.886324
Alaria esculenta	3	Kangerlussuaq	68.134950	-32.001224
Alaria esculenta	1	Maniitsoq	65.421645	-52.886324
Alaria esculenta	1	Nuuk	64.177752	-51.746300
Alaria esculenta	1	Qerrortusoq	66.916707	-53.532217
Alaria esculenta	1	South of Immikkeertikajik	69.373263	-23.331996
Alaria esculenta	2	South of Immikkeertikajik	69.379811	-23.332537
Ascophyllum nodosum	1	Maniitsoq	65.421645	-52.886324
Ascophyllum nodosum	3	Narsarsuaq	61.145833	-45.434722
Ascophyllum nodosum	1	Nuuk	64.177752	-51.746300
Ascophyllum nodosum	2	Sisimiut	66.938889	-53.672222
Ascophyllum nodosum	1	Sisimiut hospital	66.943028	-53.651677
Fucus distichus	4	Kangerlussuaq	68.192411	-32.114631
Fucus distichus	1	Maniitsoq	65.421645	-52.886324
Fucus distichus	1	Nuuk	64.177752	-51.746300
Fucus distichus	2	Sisimiut	66.938889	-53.672222
Fucus spp.	2	Ilulissat kajak club	69.220537	-51.112659
Fucus spp.	3	Sisimiut dump	66.928316	-53.673514
Fucus spp.	2	Sisimiut hospital	66.943028	-53.651677
Fucus vesiculosus	1	Maniitsoq	65.421645	-52.886324
Fucus vesiculosus	3	Narsarsuaq	61.145833	-45.434722
Fucus vesiculosus	1	Nuuk	64.177752	-51.746300
Fucus vesiculosus	1	Qerrortusoq	66.916707	-53.532217
Fucus vesiculosus	2	Sarfannguit dump	66.897536	-52.874138

Continued on next page.

Summary of Greenland seaweed samples included in the study. Coordinates in decimal degrees. (Continued from previous page)

Species	n	Location	Latitude	Longitude
Fucus vesiculosus	2	Sarfannguit factory	66.898226	-52.858431
Fucus vesiculosus	2	Sarfannguit school	66.896311	-52.857659
Fucus vesiculosus	3	Sisimiut	66.938889	-53.672222
Fucus vesiculosus	1	Sisimiut hospital	66.943028	-53.651677
Hedophyllum nigripes	1	Kangikajik	70.000763	-22.304657
Hedophyllum nigripes	2	Maniitsoq	65.421645	-52.886324
Hedophyllum nigripes	1	Nuuk	64.177752	-51.746300
Hedophyllum nigripes	1	South of Immikkeertikajik	69.373263	-23.331996
Laminaria solidungula	1	Kangerlussuaq	68.192411	-32.114631
Laminaria solidungula	2	South of Immikkeertikajik	69.379811	-23.332537
Laminaria solidungula	3	Uunartertaqartikajiip Oqqummut Kangertiva	69.330236	-24.087100
Palmaria palmata	1	Nuuk	64.177752	-51.746300
Palmaria palmata	1	Sarfannguit factory	66.898226	-52.858431
Saccharina latissima	1	Kangerlussuaq	68.134950	-32.001224
Saccharina latissima	3	Kangikajik	70.000763	-22.304657
Saccharina latissima	5	Sisimiut	66.938889	-53.672222
Saccharina latissima	2	Uunartertaqartikajiip Oqqummut Kangertiva	69.330236	-24.087100
Saccharina longicruris	1	Maniitsoq	65.421645	-52.886324
Saccharina longicruris	1	Nuuk	64.177752	-51.746300

No permits were required for the described field study as none of the locations are privately owned or protected. This study did not involve endangered or protected species.

5.3.2 Sample pre-treatment

Samples were rinsed in clean seawater at the collection site, epibiota were carefully removed, samples were frozen in clean food grade plastic bags at -20 ℃ and transported frozen to the laboratory in Denmark. Samples were freeze dried (Christ Beta 1-8, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and, for compositional comparison of different thallus parts from five algal species, thereafter manually divided into blade, midrib and stipe, see Fig 5.2. Some of the received samples of *S. longicruris* and *S. latissima* had already been divided into stipe and blade. Additional epibiota was removed at this point. However, a limited presence of some epibiota, such as small crustaceans, especially on *A. nodosum*, cannot be ruled out due to the very branched structure of this macroalga. Homogenised powders were produced in a mill (Knifetec 1095 Sample Mill, FOSS, Hillerød, Denmark). Surplus sample material was saved for future studies.

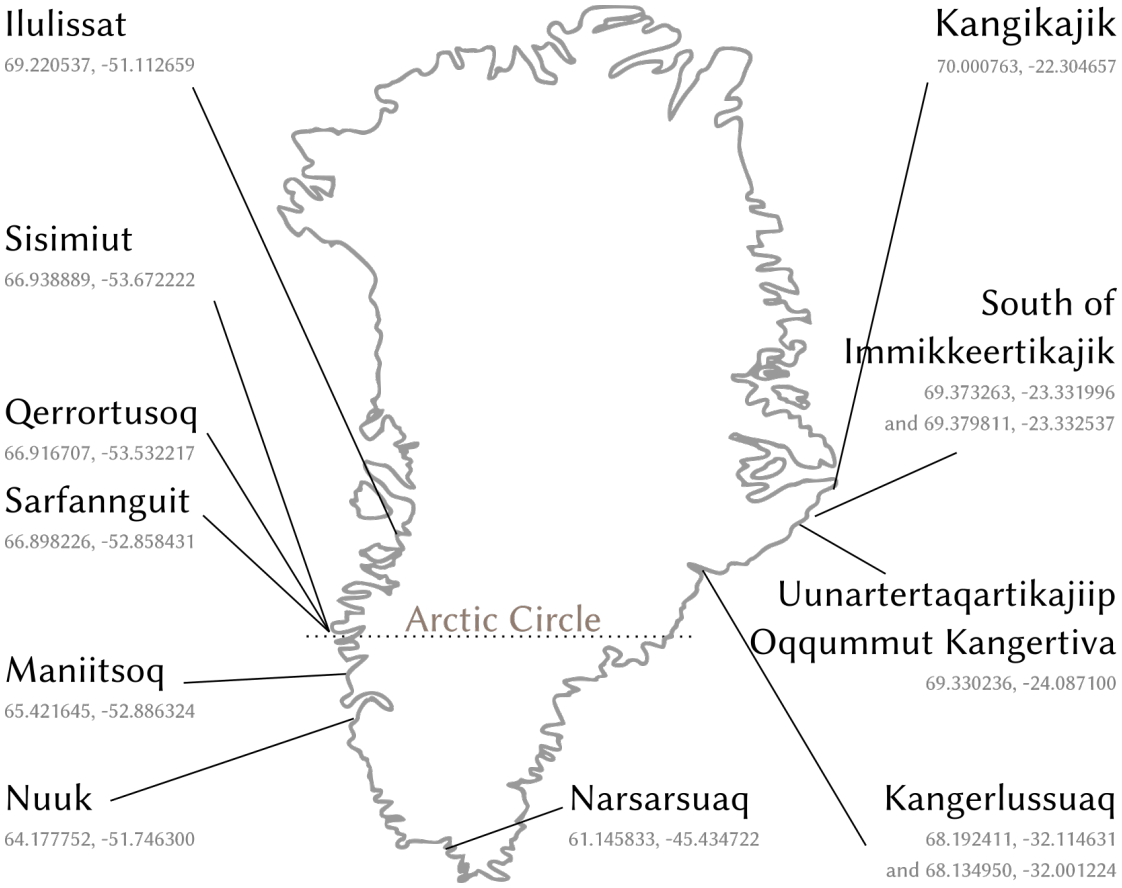

Figure 5.1: Sampling locations in Greenland with coordinates in decimal degrees (latitude, longitude). For Sarfannguit, coordinates are given for one central location (fish factory), specific coordinates for all three sampling sites are provided in Table 5.1.

5.3.3 Analytical methods

All chemicals were of *pro analysi* quality or better, and all sample tubes were of inert quality to avoid contamination. All plastic tubes were new and all quartz digestion vessels were cleaned by microwave-assisted heating with concentrated nitric acid (HNO_3) (PlasmaPure, SCP Science, Courtaboeuf, France), and subsequent thorough rinsing with ultrapure water (18.2 MΩ at 25 ℃, maximum 2 ppb total organic carbon, Milli-Q Integral 5 Water Purification System, Merck KGaA, Darmstadt, Germany).

This study was carried out using the principles in a modified and combined version of two reference methods, EN 13805:2014 [71] and EN 15763:2009 [73], for the determination of all elements except iodine.

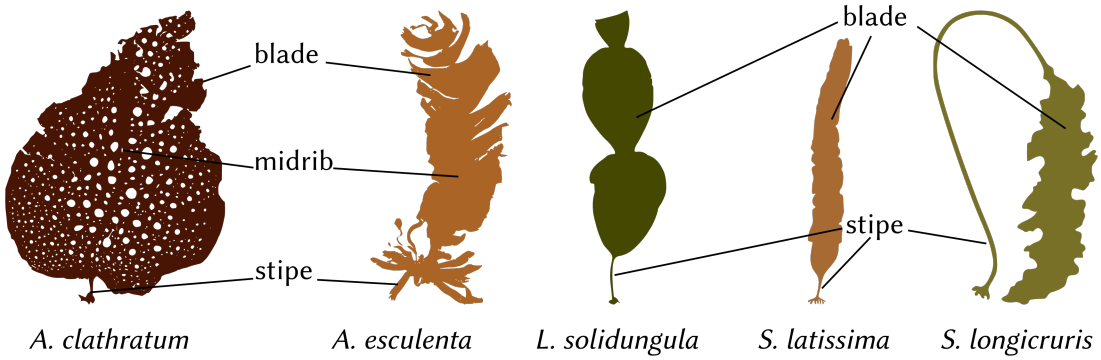

Figure 5.2: Diagram showing the individually examined algal thallus parts of Greenland seaweeds: *A. clathratum*, *A. esculenta*, *L. solidungula*, *S. latissima*, and *S. longicruris*.

An aliquot of 0.2 g seaweed powder was weighed into pre-weighed quartz digestion vessels to the nearest 1 mg. Two millilitre ultrapure water, followed by 4 mL concentrated HNO_3 were added to the sample. The samples were digested in a microwave reaction system (Multiwave 3000, Anton Paar GmbH, Graz, Austria). Following digestion, the samples were transferred to pre-weighed 50 mL centrifuge tubes, diluted to about 25 mL with ultrapure water and reweighed. Sample aliquots were further diluted (5, 100 and 1000 times) in 2% HNO_3 to a respective element concentration between 0 and 400 ng mL^{-1} for analysis on the inductively coupled plasma mass spectrometry (ICP-MS) instrument. For each batch of 16 samples, two samples were determined in duplicates, one procedural blank (only ultrapure water and HNO_3) and one certified reference seaweed sample (NMIJ CRM 7405-a; Trace elements and arsenic compounds in seaweed (Hijiki, *Sargassum fusiforme*), National Metrology Institute of Japan, Tsukuba, Japan)) were processed and analysed alongside the rest of the samples. As internal standard, a mixture of Bi, In and Rh was prepared from single element calibration standards (PlasmaCal, SCP Science). A calibration curve from 0 to 400 ng mL^{-1} was prepared for all elements from single element calibration standards (PlasmaCAL). The samples were analysed on an 8900 ICP-MS Triple Quad (Agilent Technologies, Santa Clara, USA) equipped with an SPS4 Autosampler (Agilent Technologies).

For iodine, a modified version of the EN 15111:2007 [72] reference method was used. An aliquot of 0.3 g seaweed powder was weighed into pre-weighed 50 mL centrifuge tubes to the nearest 1 mg. Five millilitre of ultrapure water were added, and thoroughly mixed. Thereafter, 1 mL 25% tetramethylammonium hydroxide (TMAH) (TMAH, 25% w/w aq. soln., Alfa AesarTM, Fisher Scientific, Waltham, Massachusetts, USA) solution was added and the samples mixed again. The samples were heated in a drying oven (Memmert UF 30, Memmert GmbH + Co. KG, Schwabach, Germany) at 90 ℃ for three hours. After 1.5 hours, the samples were removed briefly and inverted to ensure nothing stuck to the bottom. After cooling, the sam-

ples were diluted with 0.5% TMAH to approximately 50 mL and weighed again. A 5 mL aliquot of each sample was transferred to centrifuge tubes and centrifuged at 20 000 g for 5 min. The supernatant was then further diluted in 0.5% TMAH to a final iodine concentration between 0 and 100 ng mL^{-1} for analysis on the ICP-MS instrument. Four procedural blanks and two certified replicate seaweed reference samples (3232 Kelp powder *Thallus laminariae*, NIST National Institute of Standards and Technology, Gathersburg US. ID 160129) were processed along with the samples. One in every ten samples was also determined in duplicate. Tellurium (Te) (PlasmaCal, SCP Science) was used as the internal standard and a calibration curve was prepared from ultrapure iodide (Iodide 1000 µg L^{-1} Spectrascan SS11I, Ski, Norway) from 0 to 100 ng mL^{-1} iodine. The samples were analysed on an iCAP Q ICP-MS (Thermo Scientific, Bremen, Germany) equipped with an ASX-520 AutoSampler (Cetac) running Qtegra version 2.10.3324.83 (64 bit) (Thermo Scientific).

The limit of detection (LOD) and limit of quantification (LOQ) for both methods were calculated using the standard deviation (SD) obtained from repeated analysis of blank samples:

LOD respectively LOQ = SD of the blanks (ng/mL) * f * dilution factor (mL)/sample amount (g), with f = 3 for LOD and f = 10 for LOQ.

The precision was determined from duplicate analysis of the same sample. The relative standard deviation (RSD), a measure of repeatability, was calculated from duplicate samples determined at different dates by the same operator on the same equipment.

ICP-MS parameters are summarised in table A.1 (page 154).

For Na, I and P, LOD and LOQ were derived from the lowest accepted concentration of the calibration curve since the blanks were below detection limit. Relative standard deviations were in the range of 0.01% to 25%, with medians between 1.6% and 4.6% for all elements except Hg and Se with relative standard deviations up to 55% and 31%, respectively, and medians of 22% and 11%.

Assessment of normality revealed that element concentrations were not normally distributed. Therefore, element concentrations are reported as median values with median absolute deviation, and non-parametric tests were used for data analysis.

5.3.4 Portion size survey

To determine an appropriate portion size for a seaweed salad served in Denmark, we asked six restaurants in the Copenhagen area and one Danish seaweed producer about their typical serving size for a seaweed salad. Only businesses that had seaweed salads on their menu or in their online store were contacted. Queries were sent by e-mail to the contact addresses available from the respective homepages. No personal data were collected and results were anonymised.

For the purpose of calculating the exposure to beneficial and toxic elements from eating a salad prepared from Greenland seaweed, we made the following assumptions: 1) such a salad would solely consist of one species of seaweed, 2) cutting up the fresh seaweed into a salad would not change the observed concentrations and 3) dressing (if used) would constitute a

negligible part of the total weight and nutritional content.

5.3.5 Statistical analyses

For data analysis, results for those samples that had been divided into different thallus parts were pooled for analysis on species level. All data analysis was carried out with with R version 3.4.4 (2018-03-15) [181], using RStudio version 1.1.463 [193], on a x86_64-pc-linux-gnu (64-bit) platform. Data was imported via readxl [242], transformed using dplyr [243], analysed with the stats package, and visualised with ggplot2 [241]. For upload to external databases, data was exported with WriteXLS [203] or writecsv (utils package 3.4.4).

Normality was assessed with the Shapiro-Wilk test. Since preconditions for parametric tests were not met, Kendall's ranked correlation coefficient was used for pairwise element correlations and the Kruskal-Wallis test was used to compare differences between species and locations. Principal component analysis was carried out using the prcomp function (stats package 3.4.4), with centring and scaling of samples.

A confidence level of 95% was used unless otherwise noted.

5.4 Results and discussion

5.4.1 Quality assurance

Quality assurance parameters are presented in table A.2 (page 155).

5.4.2 Individual sample results

The full dataset of individual sample results is freely available online [112]. Samples with different thallus parts examined separately are connected by their sampleID to the respective powderIDs. In the present article, summarised results are presented and discussed.

5.4.3 Species comparison

Median element contents are presented in Table 5.2 and figures A.1 (page 156), A.2 (page 157), A.3 (page 158) and A.4 (page 159). The most abundant cations were K>Na>Ca>Mg (3.79 g kg^{-1} to 108 g kg^{-1} freeze dried weight). Schiener and colleagues [198] found the same sequence in an investigation that included among other seaweeds species, *S. latissima* and *A. esculenta*. These four light metal cations are also the most abundant cations in seawater, with Na>Mg>Ca>K [220]. In seaweeds, these cations are gradually replaced by heavier divalent metal ions such as Cu from the seawater [46], during the continued growth of the algae [16].

Table 5.2: Median content ± median absolute deviation of elements in Greenland seaweed samples, freeze dried weight.

Species	n	As (mg kg^{-1})	Ca (g kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})
A. clathratum	3	46.1 ± 17.2	30.4 ± 7.1	0.208 ± 0.113	5.75 ± 1.18	4.51 ± 1.55
A. esculenta	9	33.0 ± 14.8	18.2 ± 2.7	1.32 ± 0.66	7.07 ± 7.05	2.70 ± 1.54
A. nodosum	8	29.8 ± 2.3	14.4 ± 2.5	0.293 ± 0.138	1.17 ± 0.11	5.65 ± 4.28
F. distichus	8	40.1 ± 5.9	14.0 ± 1.0	0.952 ± 0.373	5.78 ± 5.85	19.1 ± 25.1
Fucus spp.	7	26.6 ± 6.6	14.4 ± 1.8	0.826 ± 0.233	2.56 ± 1.17	4.32 ± 0.40
F. vesiculosus	16	33.3 ± 7.6	13.4 ± 1.4	1.42 ± 1.01	1.29 ± 0.76	2.32 ± 1.43
H. nigripes	5	63.1 ± 27.1	14.1 ± 3.0	0.168 ± 0.169	1.70 ± 1.54	2.85 ± 1.97
L. solidungula	6	47.6 ± 13.1	10.6 ± 0.8	0.134 ± 0.036	1.61 ± 0.52	9.79 ± 10.25
P. palmata	2	6.93 ± 1.97	3.79 ± 2.73	0.600 ± 0.271	0.657 ± 0.177	5.71 ± 3.57
S. latissima	11	45.2 ± 12.1	10.8 ± 2.8	2.96 ± 1.08	1.89 ± 1.84	1.72 ± 1.33
S. longicruris	2	61.9 ± 5.1	15.4 ± 1.3	1.25 ± 0.24	1.14 ± 0.58	1.40 ± 0.28

Species	n	Fe (mg kg^{-1})	Hg (mg kg^{-1})	I (mg kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})
A. clathratum	3	492 ± 368	< 0.023*	280 ± 46	37.5 ± 20.5	6.25 ± 0.65
A. esculenta	9	306 ± 303	< 0.078**	502 ± 307	78.6 ± 31.3	9.20 ± 2.01
A. nodosum	8	82.2 ± 25.8	< 0.078**	670 ± 162	20.5 ± 0.8	9.28 ± 1.42
F. distichus	8	702 ± 834	< 0.023*	212 ± 43	33.1 ± 2.4	9.41 ± 1.26
Fucus spp.	7	365 ± 342	< 0.023*	234 ± 44	36.7 ± 4.1	8.92 ± 1.05
F. vesiculosus	16	119 ± 120	< 0.023* *****	188 ± 74	25.5 ± 3.2	8.52 ± 0.97
H. nigripes	5	171 ± 111	< 0.078**	3323 ± 742	90.6 ± 30.8	6.65 ± 0.55
L. solidungula	6	406 ± 166	< 0.078**	4478 ± 1812	93.1 ± 29.6	4.92 ± 0.59
P. palmata	2	131 ± 94	< 0.023*	113 ± 90	75.7 ± 18.2	4.63 ± 1.22
S. latissima	11	124 ± 139	< 0.078**	3124 ± 927	59.1 ± 18.2	7.03 ± 2.07
S. longicruris	2	183 ± 169	< 0.023*	1466 ± 702	108 ± 55	7.37 ± 1.24

Species	n	Mn (mg kg^{-1})	Na (g kg^{-1})	Ni (mg kg^{-1})	P (g kg^{-1})	Pb (mg kg^{-1})
A. clathratum	3	13.5 ± 9.1	28.2 ± 2.1	3.35 ± 1.31	1.48 ± 0.59	0.337 ± 0.162
A. esculenta	9	9.60 ± 8.30	48.2 ± 11.0	3.52 ± 3.31	2.18 ± 1.42	0.474 ± 0.557
A. nodosum	8	11.0 ± 3.9	37.7 ± 4.5	0.992 ± 0.133	1.06 ± 0.25	0.111 ± 0.110
F. distichus	8	36.5 ± 19.3	44.8 ± 4.6	7.80 ± 1.42	1.26 ± 0.04	0.243 ± 0.066
Fucus spp.	7	26.8 ± 8.6	38.5 ± 5.2	5.99 ± 1.74	1.76 ± 0.37	1.59 ± 2.18
F. vesiculosus	16	34.3 ± 24.5	40.1 ± 6.6	3.33 ± 0.85	1.00 ± 0.33	0.101 ± 0.064
H. nigripes	5	4.69 ± 1.86	31.5 ± 3.9	1.40 ± 1.18	2.39 ± 0.39	0.158 ± 0.133
L. solidungula	6	7.85 ± 5.05	24.5 ± 4.1	1.12 ± 0.63	2.04 ± 1.17	0.329 ± 0.266
P. palmata	2	5.95 ± 2.85	35.0 ± 12.8	3.84 ± 2.28	2.54 ± 0.60	0.251 ± 0.274
S. latissima	11	4.18 ± 3.09	36.8 ± 10.1	1.14 ± 0.85	2.24 ± 1.17	0.207 ± 0.218
S. longicruris	2	3.35 ± 1.71	34.9 ± 3.4	0.783 ± 0.078	2.11 ± 0.62	0.641 ± 0.875

* LOD, ** LOQ, *** n=2, **** n=4

*****Hg was detected in a single sample of *F. vesiculosus* at levels below LOQ. ****** Se was detected in a single sample of of *F. vesiculosus* at 0.132 mg/kg dry weight.

Continued on next page.

Median content ± median absolute deviation of elements in Greenland seaweed samples, freeze dried weight. (Continued from previous page)

Species	n	Se (mg kg^{-1})	Zn (mg kg^{-1})
A. clathratum	3	0.227 ± 0.089***	20.2 ± 12.7
A. esculenta	9	0.159 ± 0.044	19.7 ± 9.8
A. nodosum	8	< 0.111**	23.9 ± 11.4
F. distichus	8	0.142 ± 0.003****	17.4 ± 6.0
Fucus spp.	7	0.173 ± 0.090***	75.5 ± 37.9
F. vesiculosus	16	< 0.111** ******	16.6 ± 12.0
H. nigripes	5	< 0.111**	30.9 ± 10.3
L. solidungula	6	< 0.111**	12.3 ± 4.5
P. palmata	2	< 0.111**	64.1 ± 56.0
S. latissima	11	< 0.111**	18.5 ± 15.2
S. longicruris	2	< 0.111**	21.3 ± 7.1

* LOD, ** LOQ, *** n=2, **** n=4; *****Hg was detected in a single sample of *F. vesiculosus* at levels below LOQ. ****** Se was detected in a single sample of of *F. vesiculosus* at 0.132 mg/kg dry weight.

The next most abundant cation was Fe with 82.2 mg kg^{-1} to 492 mg kg^{-1}, while the most abundant other elements were P with 1.00 g kg^{-1} to 2.54 g kg^{-1} and I with 113 mg kg^{-1} to 4478 mg kg^{-1}. For all species, Hg concentrations were below the LOQ (0.078 mg kg^{-1}) or below the LOD (0.023 mg kg^{-1}). For most species, Se was below the LOQ (0.111 mg kg^{-1}), except for *A. clathratum*, *A. esculenta*, *F. distichus* and *Fucus* spp., which ranged from 0.142 mg kg^{-1} to 0.227 mg kg^{-1}. All other elements (As, Cd, Cr, Cu, Mn, Ni, Pb, Zn) were in the range of 0.101 mg kg^{-1} to 75.5 mg kg^{-1}.

Palmaria palmata, the only red seaweed studied, deviated mainly with respect to its lower content of As, Ca and I (6.93 mg kg^{-1}, 3.79 g kg^{-1} and 113 mg kg^{-1}, respectively) compared to the brown seaweeds investigated. This is in accordance with previous studies [98, 136, 148, 189].

The high iodine contents (1466 mg kg^{-1} up to 4478 mg kg^{-1}) found in Laminariaceae (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*) are in accordance with previous reports [52, 98, 189]. For *Laminaria digitata*, another member of the Laminariaceae family, Küpper and colleagues [119] proposed that iodine, in the accumulated form of iodide, functions as an inorganic antioxidant. Ye and colleagues [247] also found iodoperoxidases in *Saccharina japonica*, another member of Laminariaceae. We therefore theorize that the four members of Laminariaceae studied here also possess iodoperoxidases, leading to the high observed accumulation of iodine.

Riget, Johansen and Asmund [185] reported concentrations of selected elements (As, Cd, Cr, Cu, Fe, Pb, Zn) in *A. nodosum* and *F. vesiculosus* collected in the Nuuk area between 1980 and 1990. The major differences between their findings, and the findings of this study were increased Fe and Zn concentrations for both species. For *A. nodosum*, they reported Fe concentrations of 16 to 43 mg kg^{-1}, in this study we found 140 mg kg^{-1}, and Zn concentrations were reported as 6.6 to 10.7 mg kg^{-1}, while in this study we found 58.1 mg kg^{-1}. For *F. vesiculosus*, Fe

concentrations of 33 to 77 mg kg^{-1} were reported by Riget, Johansen and Asmund [185], while in this study we found 133 mg kg^{-1} and they reported Zn concentrations of 7.2 to 10.2 mg kg^{-1} compared to the 50.6 mg kg^{-1} in this study. The most likely explanation could be the difference in sampling: Riget, Johansen and Asmund [185] collected five samples of growing tips, while in this study the entire thallus was analysed, and samples were pooled so this study only reports one result per species from Nuuk. Another explanation could be the increased human and industrial activity in the area since their study - Nuuk has nearly doubled in size, from around 9 000 inhabitants to close to 18 000 inhabitants [93]. This is supported by another study from Greenland investigating the influence of increased human activity [213] by monitoring Cr concentrations in indicator organisms, in this case mining. *Fucus vesiculosus* was used as one of the monitoring species, and Cr concentrations increased from 0.4 mg kg^{-1} dry weight prior to mining operations, up to 2.62 mg kg^{-1} dry weight during active operations of an open-pit mine in Southern Greenland. However, the Cr concentrations found in this study had not increased in a comparable manner to what was observed for the mining operation.

The presence of overall tendencies in element content or fingerprint per algal family were assessed by PCA, as presented in Fig 5.3. Both Hg and Se were excluded from the analysis due to the very low observed concentrations, which could not be quantified for the majority of samples. Fucaceae (*A. nodosum*, *F. distichus* and *F. vesiculosus*), characterised by a higher content of Mg, Mn and Ni, could clearly be distinguished from Laminariaceae (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*), which had a higher content of especially iodine, but also K and P. Alariaceae (*A. esculenta*) could not be distinguished from the other families with this method, and for Agaraceae (*A. clathratum*) and Palminariales (*P. palmata*), the low sample number of three and two samples, respectively, precluded analysis in this manner. To the best of the authors knowledge, this is the first PCA presented in the literature of this specific combination of species. It is interesting to note that Laminariaceae, known for their high contents of iodine, could be distinguished from Fucaceae based mainly on their iodine content.

We also used PCA to investigate the influence of nearby human settlement size, based on the content of elements associated with anthropogenic contamination (Cd, Cr, Cu, Pb and Zn). However, there was no clear correlation evident.

5.4.4 Thallus part comparison

For five species, a limited number of samples were divided into different parts, in particular blade, midrib and stipe: *A. clathratum* and *A. esculenta*, or blade and stipe: *L. solidungula*, *S. latissima* and *S. longicruris* (see Fig 5.2. for a schematic representation). Selected elements (As, Cd, Fe, I, K and Pb) are presented in Fig 5.4.

Concentrations of As were higher in stipes than in blades for *S. latissima* (Fig 5.4, panel A), and similarly, K concentrations in *S. latissima* and *S. longicruris* (Fig 5.4, panel E). A possible explanation for this could be that metal(loid)s (such as As, Cd, Hg, K and Pb) are stored associated with biopolymers [46], and these biopolymers are differently distributed throughout the thallus. Research into the properties of alginate, with respect to divalent metal ions, from

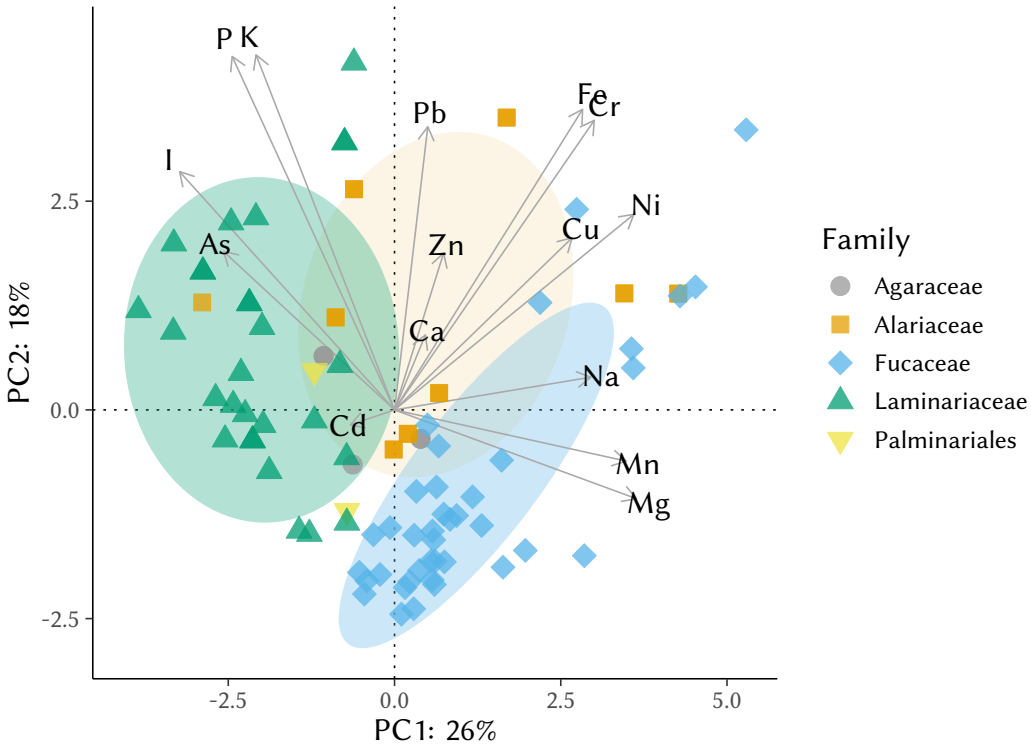

Figure 5.3: Principal component analysis of element content on family level of Greenland seaweeds. Agaraceae (*A. clathratum*), Alariaceae (*A. esculenta*), Fucaceae (*A. nodosum*, *F. distichus*, *F. vesiculosus* and *Fucus* spp.), Laminariaceae (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*) and Palminariales (*P. palmata*). Hg and Se were excluded from the analysis due to the low number of quantifiable samples. Ellipses denote 95% confidence intervals for Alariaceae, Fucaceae and Laminariaceae.

Laminara digitata and *Laminaria hyperborea* in the 1960ies also showed differences between stipe and other (nondisclosed) parts of macroalgae [91] and is supported by observations by S. Wegeberg & O. Geertz-Hansen (unpublished data).

The comparison of reports on the concentrations ($mg\,kg^{-1}$) of total As in Icelandic *A. esculenta* from the Pétursdóttir and colleagues [178] and present studies showed for: stipes (53 ± 3 and 45 ± 6); midrib (43 ± 4 and 23 ± 7); and blade (93 ± 4 and 31 ± 16) respectively. With regards to the As content ($mg\,kg^{-1}$) in Icelandic *S. latissima*, the Pétursdóttir and colleagues [178] and present studies reported for: stipes (53 ± 4 and 75 ± 32); and blade ([117 ± 9 – old frond and 116 ± 6 – young frond] and 53 ± 7) respectively.

The differences between the study of Pétursdóttir and colleagues [178] and ours could be

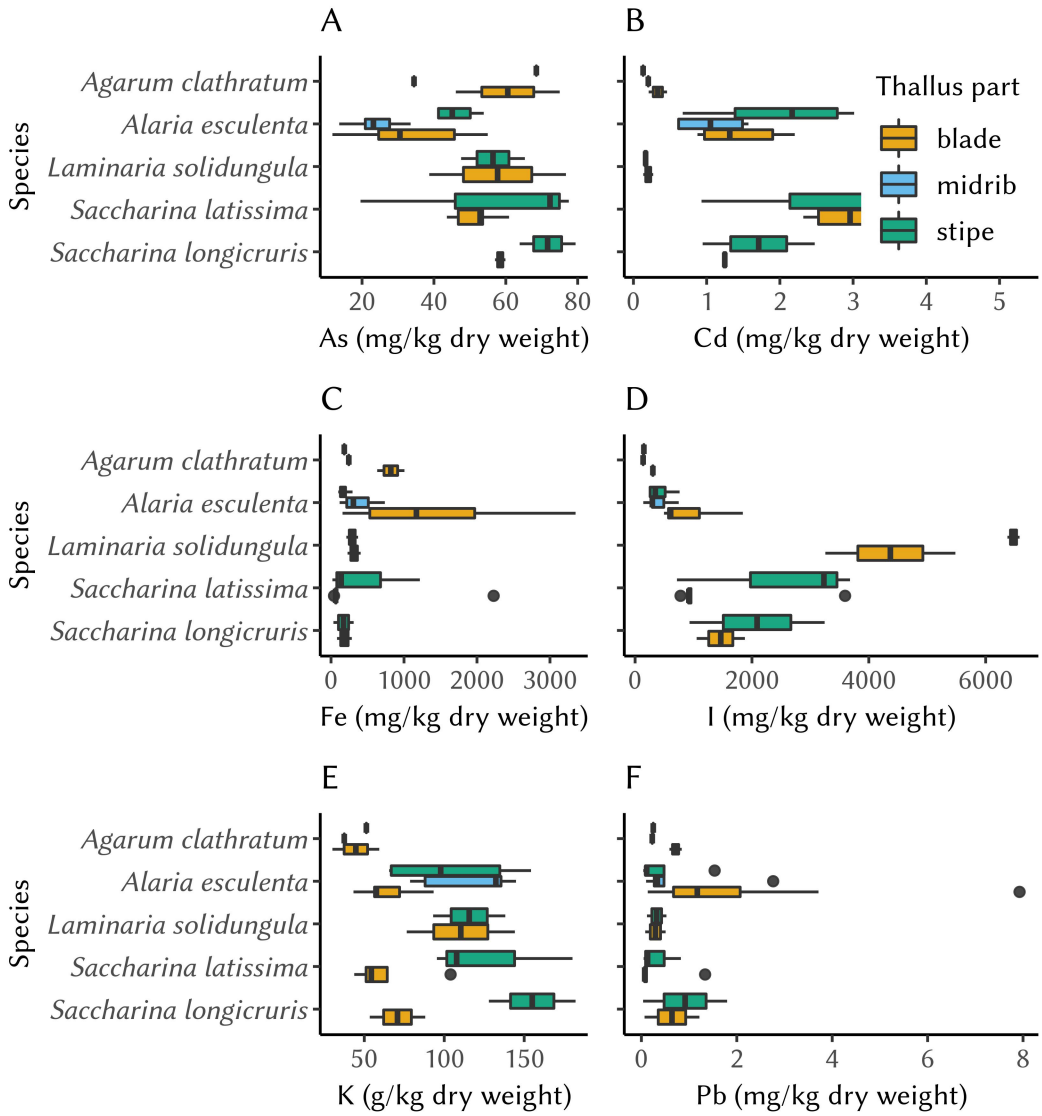

Figure 5.4: Concentrations of elements (As, Cd, I, Fe, K and Pb) in different thallus parts of Greenland seaweeds for *A. clathratum*, *A. esculenta*, *L. solidungula*, *S. latissima* and *S. longicruris*. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range. Outliers beyond the whiskers are shown as circles.

related to the time of sampling: they sampled during late winter, while the seaweeds in our study were collected during June to September. Previous studies have shown a seasonal change in nutritional composition of seaweeds (e.g. [186, 198]). Another possible explanation is the small sample size of both studies: two samples of Pétursdóttir and colleagues [178], whereas the present study reports on three stipe and five blade samples.

Ronan and colleagues [191] reported that total arsenic concentrations of both *A. nodosum* and *L. digitata* increased with the age of the thallus part, which is a probable explanation for the wide range of arsenic concentration observed in our study.

An explanation for the higher concentration of iodine in stipes compared to blades of *L. solidungula* and *S. latissima* (Fig 5.4, panel D) could be that these species possess iodoperoxidases, which are upregulated in parts of the macroalgae that are more exposed to environmental stress and pathogens, such as stipes, similarly to what Ye and colleagues [247] found for *S. japonica*. Another explanation is related to the age of the macroalgal part: while stipes are perennial, blades are annual.

Interestingly, some elements show a great variation in concentrations in the blade, such as Fe for *A. esculenta*, but not for any of the other Laminariaceae, see figure (Fig 5.4., panel C). This could be due to iron accumulating differently in older compared to younger macroalgae, or thallus parts, which have been shown to grow at different rates by Buggeln and colleagues [31].

Differences in element concentrations can also depend on where the sample is taken on the blade. This is due to the localization of meristem and thus the allocation of nutrients for growth, e.g., close to the stipe and hence close to the meristem or distally (S. Wegeberg and O. Geertz-Hansen (unpublished data on biopolymers), [198]). For *L. solidungula*, the blade generation is also significant. In the present study, neither the localisation on the blade nor the blade generation were investigated.

5.4.5 Geographic origin comparison

All samples of *F. distichus* (n = 8), *F. vesiculosus* (n = 16) and *Fucus* spp. (n = 7) were used in a pooled investigation. Figure 5.5 presents the results of the PCA, from which Hg and Se were again excluded, as previously for the species comparison. The samples can clearly be divided into Western (Maniitsoq, Nuuk, Qerrortusoq, Sarfannguit and Sisimiut), Southern (Narsarsuaq) and Eastern (Kangerlussuaq) origin. The three samples from the dump in Sisimiut illustrate the strong influence of human waste on the elemental composition of *Fucus* species. They are clearly separated along PC1 from the remaining Sisimiut samples, which include those from the hospital sewage outlet into Kangerluarsunnguaq, a bay with little water exchange. The samples from Ilulissat were collected within the city limits, where wastewater is diverted untreated into the sea. This human impact on the elemental composition is clearly reflected in the PCA, where the Ilulissat samples are grouped into the same quadrant as those from the dump in Sisimiut. By analysing many elements, it is thus possible to distinguish between locations even at small sample sizes per location. Analysis of location differences based on a single element through

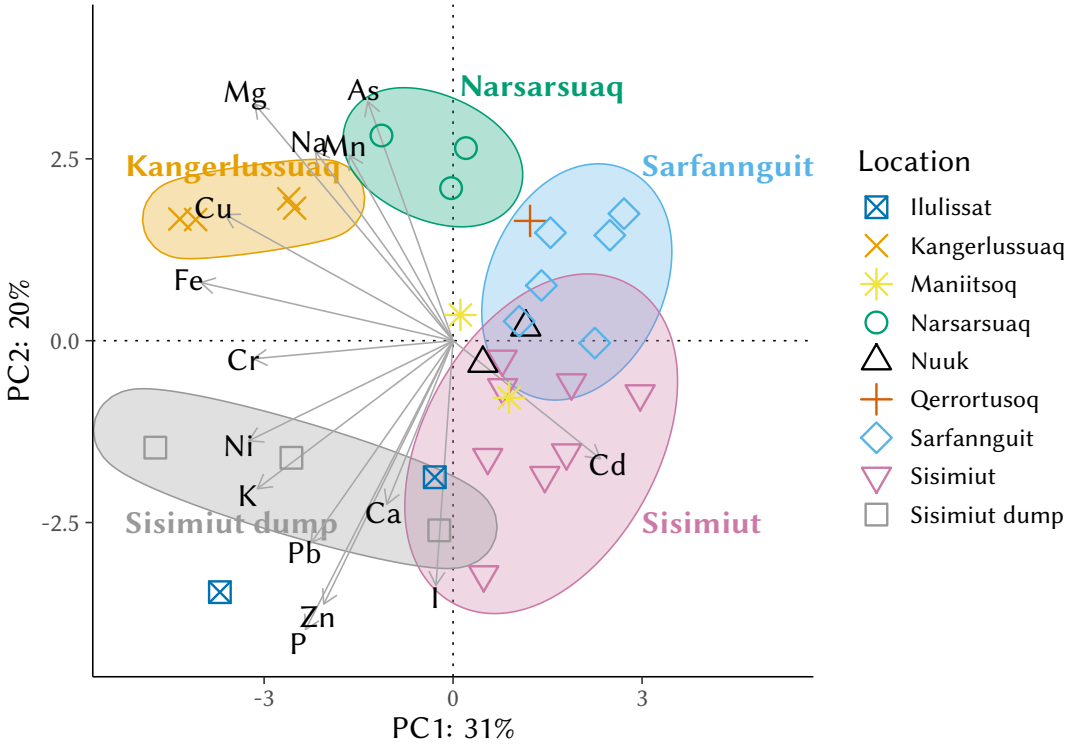

Figure 5.5: Principal component analysis of element content of Greenland seaweeds depending on geographic location. All samples of *F. distichus*, *F. vesiculosus* and *Fucus* spp. were used in a pooled investigation. Hg and Se were excluded from the analysis due to the low number of quantifiable samples. Ellipses denote locations with at least three samples.

Kruskal-Wallis testing revealed statistically significant differences ($p<0.05$) for the following elements: As, Cd, Cu, Fe, Mg, P, Pb and Zn. This is also reflected in the PCA, where these eight elements have the strongest influence on the PCs, as evidenced by the length of the arrows representing the loadings.

The observed natural variation at a given sampling site may be due to different factors, both abiotic (e.g. salinity) and biotic (e.g. fouling). These factors may lead to metabolic changes which affect growth rates and element uptake [82]. Brinza and colleagues [29] found that Zn uptake rates differed greatly between Danish and Irish *F. vesiculosus*. They explained the greater Zn uptake rates in the specimens from Denmark with differences in surface properties of the macroalgae, related to the salinity. At their collection site in the Sound, Denmark, salinity varies between 10 to 20 practical salinity units (PSU), compared to 36 PSU in Irish waters.

5.4.6 Element correlations

Figure 5.6 summarises statistically significant values of Kendall's tau coefficient for pairwise element correlations. The strongest correlations were observed between Mg-Na (Kendall's tau 0.58) and K-P (0.58), as well as Cr-Fe (0.52), Fe-Pb (0.51) and Ni-Mn (0.51). This is also reflected in the small angles between the loadings for K-P and Cr-Fe shown in figure 5.3, a sign of correlation.

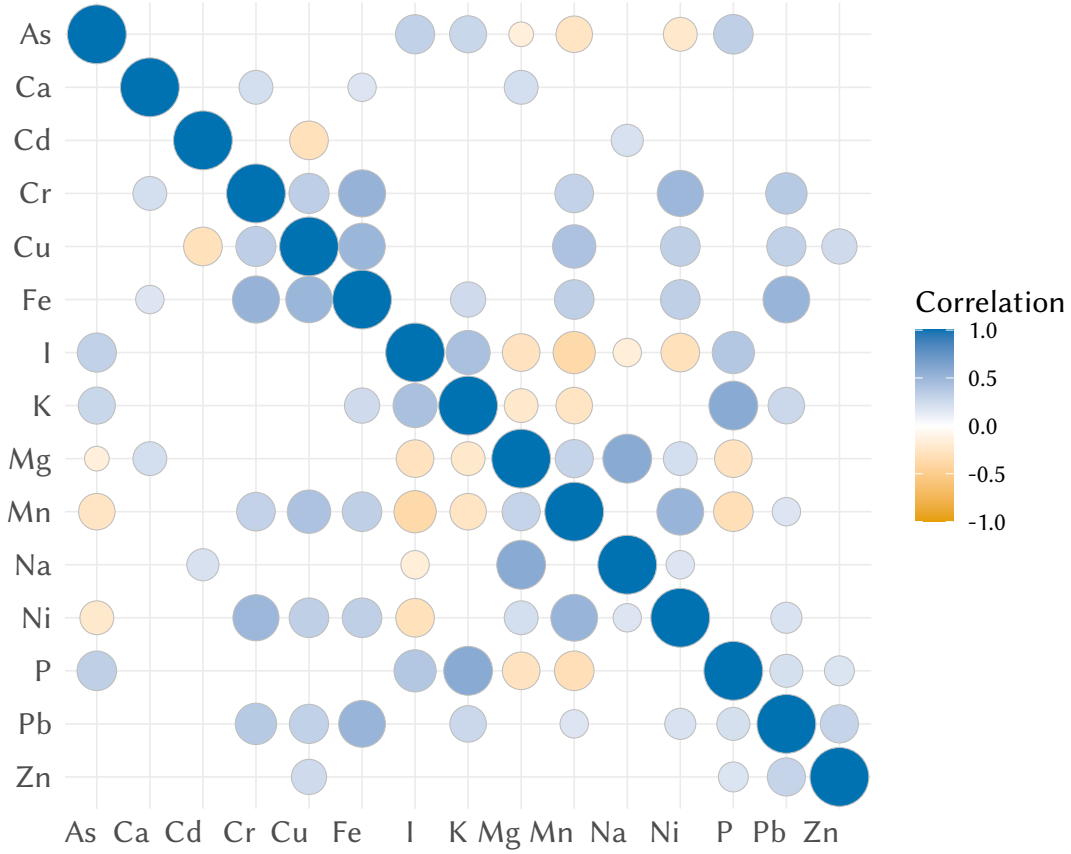

Figure 5.6: Matrix of element correlations for Greenland seaweeds, expressed as Kendall's tau coefficient. Elements are ordered alphabetically for ease of reading. Hg and Se were excluded from the analysis due to the low number of quantifiable samples. Only statistically significant correlations ($p < 0.05$) are shown.

Mg^{2+}, Na^{+} and K^{+} are some of the most common cations in seawater [220, 221]. Seaweed acquires these light metal ions from seawater, and they are, together with Ca^{2+}, indeed reported

as the main cations in seaweed biomass [46].

An explanation for the strong correlation observed between Cr and Fe can be that when Fe partially replaces Ca in the alginate matrix of the cell walls, it creates favourable binding sites for Cr, as reported by Nayak and colleagues [157].

A significant correlation between As-P (with a Kendall's tau coefficient of 0.32 in the present study), was also reported by Taylor and Jackson [224]. They reported a similar ratio of As-P in brown algae (0.015) as found in this study (0.025), which is slightly lower than the ratio found in seawater of 0.033 (As = 0.002 mg L^{-1}; P = 0.06 mg L^{-1} [221]). They argued that this similarity in ratio could be due to these two elements being taken up by the same mechanism. However, we conclude that the difference in ratios between seawater and in the seaweed suggests that seaweed is indeed able to differentiate between As and P. Arsenates and phosphates have similar chemical properties, which contributes to the toxicity of arsenates [145]. In marine algae, As(V) enters cells through phosphate transporters, while As(III) enters through the plasma membrane via aquaglyceroporins and hexose permeases [138, 145]. Most of the arsenic taken up by macroalgae is stored as arsenosugars which are considered as less toxic to humans than inorganic arsenic [224].

Miedico and colleagues [144] also reported element correlations as Pearson's coefficients on 92 samples of edible seaweeds, and despite a significant overlap in the studied elements, only three element pairs were significantly correlated ($p<0.05$) in both studies: As-Hg, Cr-Ni and Cu-Mn. These shared correlations are especially interesting since Miedico and colleagues [144] investigated different species, which furthermore originated from the Pacific. Thus these shared element pairs (As-Hg, Cr-Ni and Cu-Mn) indicate a relationship independent of macroalgae species and geographic origin.

This is supported by Desideri and colleagues [48], who reported element correlations as Pearson's coefficients on a total of 14 samples from a mixture of edible macroalgae and microalgae, some of the latter even being freshwater organisms. They did not indicate the threshold for significance, but element pairs significantly correlated in our study and those with high correlation coefficients in their study (> 0.7) were As-I, Cu-Mn and Ni-Mn.

We theorize that the shared correlations are due to chemical similarity of the element pairs, however an in-depth investigation is beyond the scope of this study.

Nutritional and food safety aspects

Table 5.3 summarises current European and Nordic guidelines on recommended daily intake levels, upper daily intake levels, maximum levels in the EU and France and toxicological guideline values for the elements investigated in our study.

Table 5.3: Current European and Nordic guidelines on recommended daily intake levels, upper daily intake levels, maximum levels in the EU and France and toxicological guideline values for the elements investigated.

Element	RI	UI	EU (mg kg^{-1} ww)	France (mg kg^{-1} dw)	Toxicological guideline value
As, inorganic	-	-	none	3[78]	3 µg kg^{-1} bw day^{-1} $BMDL_{0.5}$[244]
Ca	800 mg [164]	2.5 g [164]	-	-	-
Cd	-	-	3.0*[68]	0.5[78]	2.5 µg kg^{-1} bw week^{-1} TWI[53]
Cr	**	**	-	-	-
Cu	0.9 mg [164]	5 mg [164]	-	-	-
Fe	9 mg or 14 mg*** [164]	25 mg [164]	-	-	-
Hg	-	-	0.10**** [67]	0.1[78]	4 µg kg^{-1} bw week^{-1} inorganic Hg TWI, 1.3 µg kg^{-1} bw week^{-1} methylmercury TWI [55]
I	150 µg [69, 164]	600 µg [69, 164]	none	2000[78]	-
K	4.7 g [41]	Low potassium diet: 2 g to 3 g [41]	-	-	-
Mg	280 mg or 350 mg***** [164]	no recommendation	-	-	-
Mn	3 mg [58]	no recommendation	-	-	-

Abbreviations: Recommended daily intake (RI), upper daily intake (UI), lower confidence limit of the benchmark dose (BMDL), tolerable weekly intake (TWI), tolerable daily intake (TDI), body weight (bw), wet weight (ww), dry weight (dw).
The French regulations apply to seaweed in vegetable or condiment form.
* Food supplements consisting exclusively or mainly of dried seaweed, products derived from seaweed, or of dried bivalve molluscs.
** No recommendation given due to lack of sufficient evidence [57].
*** Lower value for men and women post menopause, higher value for women.
**** Food supplements.
***** Lower value for women and higher for men.

Continued on next page.

Current European and Nordic guidelines on recommended daily intake levels, upper daily intake levels, maximum levels in the EU and France and toxicological guideline values for the elements investigated. (Continued from previous page)

Element	RI	UI	EU (mg kg^{-1} ww)	France (mg kg^{-1} dw)	Toxicological guideline value
Na	575 mg; as salt 1.5 mg [164]	2.4 g; as salt 6 g [164]	-	-	-
Ni	-	-	none	none	2.8 µg kg^{-1} bw day^{-1} TDI [56]
P	600 mg [164]	3 g [164]	-	-	-
Pb	-	-	3.0 ****[67]	5[78]	0.50 µg kg^{-1} bw day^{-1} (developmental neurotoxicity) $BMDL_{0.1}$; 1.50 µg kg^{-1} bw day^{-1} (effects on systolic blood pressure) $BMDL_{0.1}$; 0.63 µg kg^{-1} bw day^{-1} (chronic kidney disease) $BMDL_{10}$ [54]
Se	50 µg or 60 µg ***** [164]	300 µg [164]	-	-	-
Zn	7 mg or 9 mg *****[164]	25 mg [164]	-	-	-

Abbreviations: Recommended daily intake (RI), upper daily intake (UI), lower confidence limit of the benchmark dose (BMDL), tolerable weekly intake (TWI), tolerable daily intake (TDI), body weight (bw), wet weight (ww), dry weight (dw).
The French regulations apply to seaweed in vegetable or condiment form.
* Food supplements consisting exclusively or mainly of dried seaweed, products derived from seaweed, or of dried bivalve molluscs.
** No recommendation given due to lack of sufficient evidence [57].
*** Lower value for men and women post menopause, higher value for women.
**** Food supplements.
***** Lower value for women and higher for men.

Since average seaweed consumption data for Europe has not been documented, we based our intake scenario on a typical seaweed serving size in a Danish restaurant. From the four collected responses, the portion size of a seaweed salad ranged from 20 g to 50 g, with a median of 33 g. To assess the nutritional benefits and exposure to toxic elements, we calculated element concentrations found in a 33 g single-seaweed species salad, prepared from fresh seaweed (Table 5.4). Our estimated serving size is comparable with Sá Monteiro and colleagues [194], who estimated an intake of 5 g freeze dried weight per week, which corresponds to about 30 g fresh seaweed at an estimated moisture content of 80% (based on moisture contents reported by Holdt and Kraan [98]).

In general, all investigated seaweed species are good sources of essential minerals and trace elements. One portion of a single-seaweed salad contributes with between 1% to 55% of the recommended intake for a specific element. For example, one portion of *S. latissima* salad contains 647 µg Fe, corresponding to a daily recommended intake of 5% (for women) to 7% (for men and women post menopause).

However, iodine levels were high: *P. palmata* was the only seaweed for which iodine exposure did not exceed the recommended upper daily intake of 600 µg for adults, which is in accordance with other studies [148, 189]. However, it has been shown that iodine concentrations of e.g. *S. latissima* can greatly be reduced by soaking in warm freshwater [217] or blanching in hot freshwater [161]. A recent study in Ammassalik (East Greenland) by Andersen and colleagues [4] showed that consumption of locally harvested *A. nodosum* and *Chondrus crispus* led to elevated urinary iodine excretion. After the ingestion of a 45 g seaweed meal, iodine was reported to be excreted after 36 hours [4]. The iodine richer *A. nodosum* led to higher excretion values, but overall bioavailability was about 50%. They also found that iodine excretion levels correlated to the reported frequency of seaweed consumption, with higher excretion levels for individuals reporting frequent intake of seaweed. Another study carried out in Nuuk, West Greenland, by Noahsen and colleagues [162] found that the consumption of a sushi meal comprised of a halibut maki roll with a 25 g *F. vesiculosus* salad led to increased urinary iodine excretion and elevated serum thyroid stimulating hormone (TSH), while no effect on serum estimated-free thyroxine (also known as T4) was observed. Urinary iodine excretion returned to pre-meal levels by day 2 post-meal, and TSH by day 3. They concluded that a single meal containing seaweed only had a temporary effect on the thyroid, even at high iodine concentrations in the food.

Furthermore, it is important to note that, while K is an important constituent of the human diet, for patients on a low potassium diet (2 to 3 g day^{-1}), the consumption of one seaweed salad prepared from *H. nigripes*, *L. solidungula* or *S. longicruris* would contribute with over 0.5 g K, which is up to 25% of the recommended daily intake for these patients.

None of the individual samples exceeded the EU maximum levels for Hg of 3 mg kg^{-1} wet weight, see also Table 5.4, for individual sample results see [112]. Many samples (48) exceeded the maximum levels for Cd according to French regulations. However, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) is currently evaluating whether these maximum levels will be maintained or increased to the European level, which

Table 5.4: Calculated median element content for a single-seaweed salad of 33 g wet weight, prepared from Greenland seaweed. Where applicable, percentage of recommended daily intake is indicated in parentheses. Elements exceeding recommended upper intake levels are marked in bold font.

Species	As (µg)	Ca (mg)	Cd (µg)	Cr (µg)	Cu (µg)	Fe (µg)
A. clathratum	281	185 (23)	1.27	35.1	27.5 (3)	3001 (33/21)*
A. esculenta	147	81.1 (10)	5.90	31.4	12.0 (1)	1367 (15/10)
A. nodosum	283	137 (17)	2.79	11.1	53.7 (6)	781 (10/6)
F. distichus	283	98.8 (12)	6.72	40.8	135 (15)	4951 (55/35)
F. vesiculosus	232	92.8 (12)	9.84	8.97	16.1 (2)	823 (10/6)
H. nigripes	385	85.7 (11)	1.02	10.4	17.4 (2)	1043 (12/8)
L. solidungula	254	56.6 (7)	0.716	8.61	52.3 (6)	2168 (24/16)
P. palmata	34.9	19.1 (2)	3.02	3.31	28.7 (3)	660 (7/5)
S. latissima	236	56.7 (7)	15.5	9.86	9.01 (1)	647 (7/5)
S. longicruris	375	93.4 (12)	7.57	6.93	8.50 (1)	1113 (12/8)

Species	Hg (µg)	I (µg)	K (mg)	Mg (mg)	Mn (µg)	Na (mg)
A. clathratum	NA**	**1710**	229 (5)	38.1 (14/11)***	82.3 (3)	172 (30)
A. esculenta	NA	**2243**	351 (8)	41.1 (15/12)	42.9 (2)	215 (37)
A. nodosum	NA	**6367**	158 (4)	88.2 (33/25)	105 (3)	358 (62)
F. distichus	NA	**1498**	234 (5)	66.4 (24/19)	258 (9)	316 (55)
F. vesiculosus	NA	**1305**	178 (4)	59.2 (21/17)	238 (8)	279 (49)
H. nigripes	NA	**20276**	553 (12)	40.6 (15/12)	28.6 (1)	192 (33)
L. solidungula	NA	**23901**	497 (11)	26.3 (9/8)	41.9 (1)	131 (23)
P. palmata	NA	571	381 (8)	23.3 (8/7)	29.9 (1)	176 (31)
S. latissima	NA	**16339**	309 (7)	36.8 (13/11)	21.8 (1)	192 (33)
S. longicruris	NA	**8897**	655 (14)	44.7 (16/13)	20.3 (1)	211 (37)

Species	Ni (µg)	P (mg)	Pb (µg)	Se (µg)	Zn (µg)
A. clathratum	20.4	9.01 (2)	2.05	1.389 (3/2)***	124 (2/1)***
A. esculenta	15.7	9.75 (2)	2.12	0.710 (1/1)	87.8 (1/1)
A. nodosum	9.43	10.0 (2)	1.06	NA	227 (3/2)
F. distichus	55.0	8.92 (2)	1.71	0.999 (2/2)	123 (2/1)
F. vesiculosus	23.1	6.98 (1)	0.701	NA	115 (2/1)
H. nigripes	8.51	14.6 (2)	0.964	NA	189 (3/2)
L. solidungula	5.96	10.9 (2)	1.76	NA	65.4 (1/1)
P. palmata	19.3	12.8 (2)	1.26	NA	323 (5/4)
S. latissima	5.96	11.7 (2)	1.08	NA	96.7 (1/1)
S. longicruris	4.75	12.8 (2)	3.89	NA	130 (2/1)

* Lower value for men and women post menopause, higher value for women.
** Concentration below limit of quantification, see also Table 5.2.
*** Lower value for women and higher for men.

was not exceeded by any sample. Only two samples exceeded the French limit for Pb, but none

exceeded the European limit for Pb. Many samples of Laminariaceae (*H. nigripes*, *L. solidungula*, *S. latissima*, *S. longicruris*) exceeded the French regulation maximum level for iodine.

The content of total arsenic is listed for future reference, since the content of inorganic arsenic, for which there exist toxicological guideline values, was not quantified in this study.

5.5 Conclusion

In this study, 77 samples of ten Greenland seaweed species were collected and analysed for the content of 17 elements.

The element profiles varied between species, and species from the same family tended to have similar profiles. For those species where different parts of the thallus were investigated, the element concentrations varied between different parts, in accordance with other studies. The results from the thallus part analysis of stipe, rib or blade can be used to select or discard specific seaweed parts, depending on desired high or low concentrations of specific elements.

Elements associated with anthropogenic contamination showed no clear trend with human settlement size. Broad geographic differentiation, based on element profile, was possible for *Fucus* species. However, the geographic identification was obfuscated in the case of Sisimiut and Ilulissat, for samples collected close to waste discharge. The strong influence of human waste on the elemental profile means one should refrain from harvesting close and downstream to waste discharge into the sea, even though current European limits for toxic elements were not exceeded.

Iodine contents were very high in some species of the Laminariaceae, which limits consumption of untreated raw macroalgae according to recommendations on daily intake. However, studies on washing and blanching treatments of seaweeds from other areas show that these treatments are very effective in iodine reduction, while maintaining a good nutritional profile. Recent studies in Greenland furthermore suggest that bioavailability of iodine from seaweed might be as low as 50%, and that intake of a single meal containing seaweed only had a temporary effect on the thyroid.

Future studies should focus on the influence of post-harvest treatments prior to consumption, such as drying, blanching or fermentation, on the nutritional profile of seaweeds from Greenland.

Furthermore, a more detailed investigation of seaweeds from different areas and substratum will help to elucidate geographic differences.

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Author contributions

CRediT (Contributor Roles Taxonomy) author contributions
KJK - Conceptualisation, data curation, formal analysis, funding acquisition, investigation, project administration, visualisation, writing - original draft
LTH - Conceptualisation, funding acquisition, project administration, resources, supervision, writing - review & editing
PEJ - Conceptualisation, funding acquisition, project administration, supervision, writing - review & editing
SW - Funding acquisition, resources, supervision, writing - review & editing
OG - Funding acquisition, resources, supervision, writing - review & editing
JJS - Formal analysis, resources, supervision, writing - review & editing

6 Fatty acids, amino acids, bioactive components, dry matter and ash

Publication information

Nutritional composition of ten seaweeds from Greenland

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6.1 Abstract

Seaweeds from Greenland constitute a promising and sustainable resource of foods and food ingredients. However, knowledge of the nutritional composition of different seaweed species found in Greenland is lacking. This study investigated ten species of probable future commercial interest from various locations: *Agarum clathratum*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus distichus*, *Fucus vesiculosus*, *Hedophyllum nigripes*, *Laminaria solidungula*, *Palmaria palmata*, *Saccharina latissima* and *Saccharina longicruris*. Dry matter and ash content, fatty acids, amino acids, and antioxidant activity (total phenolic content and radical scavenging activity) were determined in 50 samples in total. Nutritional profiles differed among species, but the fucoids (*A. nodosum*, *F. distichus* and *F. vesiculosus*) showed similar trends, regardless of their geographical origin. The fucoid species and *A. clathratum* showed the highest antioxidant activity and had the highest content of important long-chain unsaturated fatty acids, although, eicosapentaenoic acid (EPA, 20:5 (n-3)) was highest in *P. palmata*. The latter species also harboured the highest content of protein and total aspartic acid and glutamic acid, which in their free form are responsible for umami taste. In conclusion, the ten species investigated are promising as food items, and show a potential for use as functional food ingredients.

6.2 Introduction

Marine macroalgae, also referred to as seaweeds, have lately gained increased international interest as a potential resource of nutritious and healthy food items. While many species of seaweeds are a common component of the Asian cuisines, in the Nordic countries, they have traditionally been consumed in limited amounts [149]. In Greenland, seaweeds have historically been part of the traditional diet of the Inuit [47] and continue to a limited extent to be part of the modern diet [4]. The revived interest has resulted in seaweeds being identified as a sustainable income source, growing business and opportunity for local food production in the Northern Periphery and Arctic region of Northern Europe, which includes Greenland [165]. This region is sparsely populated and features suitable marine areas for harvest of wild stocks as well as cultivation of seaweed.

To assess the suitability of seaweeds as food items or ingredients, it is crucial to map their nutritional content. This is especially important regarding the European Union, where foods not consumed to a significant degree before a cut-off date in 1997 fall under the novel foods regulation and require certification before they may be put onto the market [74]. Seaweeds display a wide variability in their nutritional composition, which is affected by e.g., species, location, and season [98]. Major constituents in red and brown seaweeds from Northwestern Europe are ash (7 to 44 % of dry weight (dw) [98]), lipids (2 to 8 % dw [227]), proteins (1 to 44 % dw [98]) and carbohydrates (32 to 60 % [98]).

Large brown seaweeds are utilized industrially for their alginate as a food additive (e.g., as a stabilizer), but in recent years some seaweeds have been identified as promising sources of natural antioxidants, e.g., *Fucus vesiculosus* Linnaeus 1753, as a functional food ingredient [40, 94]. The demand for natural antioxidants is driven by the food and cosmetics industry which want to replace synthetic antioxidants with natural alternatives to be able to clean label their products [102]. Antioxidants are often summarily investigated as total phenolic activity (TPC) or through activity assays.

The 2013 FAO Expert consultation on dietary protein quality evaluation in human nutrition recommends that the quality of proteins should be determined based on the true ileal digestibility of each amino acid [77], and the use of the Digestible Indispensable Amino Acid Score (DIAAS). For regulatory purposes excluding infant formulas, the Food and Agriculture Organization of the United Nations [77] recommends the following scoring pattern for amino acids (expressed as mg g^{-1} protein requirement): 20 histidine, 32 isoleucine, 66 leucine, 57 lysine, 27 sulphur amino acids, 52 aromatic amino acids, 31 threonine, 8.5 tryptophan and 43 valine. We could not find any published studies on DIAAS in seaweeds, and Ho and Redan [96] blame this on the absence of standardized data on ileal digestibility of algal amino acids.

Meanwhile, to provide a protein quality estimator, studies investigating seaweed nutritional profiles without a digestibility essay commonly report amino acid (chemical) scores (AAS) or protein chemical scores [9], which are often based on the outdated protein digestibility corrected amino acid score (PDCAAS) approach [77] and calculated from a different scoring pattern than the 2013 recommendation from the FAO described above. Holdt and Kraan [98] reported amino acid scores of 31 for kelp species (Laminaria and Saccharina spp.) and Astorga-España et al. [9] reported protein chemical scores of 67 to 131 % (*Enteromorpha* and *Adenocystis* genera). These scores are still valuable since they may be useful in the preselection of seaweed species for ileal digestibility assays.

Three amino acids or their derivatives (glutamic acid, aspartic acid, and alanine) have been identified to contribute to the umami flavour [149] and may be found in elevated concentrations in seaweed. Among red seaweed species, one Nordic species, *Palmaria palmata* (Linnaeus) Weber & Mohr 1805, is especially praised for its umami flavour, for which it has become popular in the New Nordic Cuisine [148, 149]. In *P. palmata*, up to 30 % of the total amino acid content consists of these umami-enhancing amino acids [148].

Another quality indicator of a food item is its fatty acid profile. Seaweeds are a good source of long chain polyunsaturated fatty acids (LC- PUFA) [98]. An adequate intake of n-3 LC-PUFA is

associated with decreased risk of cancer, cardiovascular diseases, depression, and mental illness and benefits the development of the nervous system [208, 210]. Especially the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are of interest because humans need to take them up with their diet [210]. Simopoulos [209] also found that a high n-6/n-3 ratio was associated with both overweight and obesity, while a diet with a balanced ratio decreased weight gain and obesity. Marinho et al. [139] found that up to 50 % of all fatty acids were PUFA in Danish *Saccharina latissima* (Linnaeus) C.E. Lane & C. Mayes Druel & G.W. Saunders 2006, however, fatty acids only make up about 2 % dw in *Saccharina* species [98]. *Fucus* spp., *Ascophyllum* sp. and *P. palmata* may contain fatty acids at up to 4 % dw [98].

As summarized above, research into Nordic seaweeds for human consumption has provided an increasingly well described outline of their constituents. The pristine waters and large seaweed forests of Greenland may harbour a treasure of sea-vegetables with good nutritional profiles, but knowledge on Greenland seaweeds is scarce and may hamper future exploitation. To address this, the present study focused on elucidating the nutritional profiles of ten seaweed species from a range of locations in Greenland to prospect for future utilization of the resource. We hypothesise that the content of nutrients in Greenland seaweed species depends on species and geographic origin. The species have been chosen because they are currently consumed in Greenland or other Nordic countries (*Alaria esculenta* (Linnaeus) Greville 1830, *Ascophyllum nodosum* (Linnaeus) Le Jolis 1863, *F. vesiculosus*, *P. palmata*, *S. latissima*, *Saccharina longicruris* (Bachelot de la Pylaie) Kuntze 1891), might be consumed in the future (*Fucus distichus* Linnaeus 1767, *Hedophyllum nigripes* (J. Agardh) Starko & S.C. Lindstrom & Martone 2019 and *Laminaria solidungula* J. Agardh 1868) or are a candidate for extraction of bioactive components (*Agarum clathratum* Dumortier 1822). Of these species, the fucoids (*A. nodosum*, *F. distichus* and *F. vesiculosus*) and *P. palmata* are easily accessible and identifiable for collection from the shore. The specific objectives of this study were to 1) investigate the content of dry matter, ash, protein, essential amino acids, amino acid composition and score, as well as fatty acid composition, and to assess the antioxidant activity in a range of Greenland seaweed species, 2) compare the nutritional composition between species and evaluate possible patterns in related species and locations, and 3) identify species with promising levels of antioxidant activity.

6.3 Materials and methods

6.3.1 Samples, sampling locations and sample pre-treatment

The sampling protocol has been described in our previous publication on element concentrations in these ten species [114] (see section 5.3.1, page 28). The present study was carried out on a representative subset of samples from the previous study. Sample date, location and species highly depended on availability and possibility to sample on site, and therefore variations are seen in replicates and species from specific areas (see supplementary table 1). Since it was not possible to sample continuously throughout the ice-free period from a specific sample site, this

study does not attempt to make any claims about seasonal differences. Briefly, 50 samples were collected in Greenland from different geographical areas during June to September in 2017 and 2018 and encompassed the following species (n= number of samples): *Agarum clathratum* (3), *Alaria esculenta* (8), *Ascophyllum nodosum* (6), *Fucus distichus* (5), *Fucus vesiculosus* (9), *Hedophyllum nigripes* (4), *Laminaria solidungula* (5), *Palmaria palmata* (2), *Saccharina latissima* (6) and *Saccharina longicruris* (2). Of these ten species, *P. palmata* was the only red seaweed while the other nine species are classified as brown seaweeds, which can be divided into fucoids (*A. nodosum*, *F. distichus* and *F. vesiculosus*) and kelp species (*A. clathratum*, *H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*). Algal samples were either harvested manually or by a rake, developed for harvesting of submerged vegetation, placed in plastic bags and frozen at -18 ℃ within 24 hours of harvest. Samples were transported frozen (-18 ℃) to the National Food Institute, DTU, Kgs. Lyngby, Denmark, where the seaweed was processed into freeze dried powders. Except for holdfasts, the entire specimen was homogenised, due to the lack of enough sample material to investigate thallus parts.

6.3.2 Analytical methods

Dry matter and ash

Dry matter was determined on freeze dried powders in duplicate by drying at 102 to 105 ℃ for 20 ± 1 hours [7]. Ash content was determined by incineration in a muffle furnace at 600 ℃ for 20 ± 2 hours [6, 131].

Fatty acids

Lipids were extracted with the Bligh and Dyer method [21] with a reduced amount of solvent [89]. The lipids were transformed to fatty acid methyl esters (FAME) according to the AOCS method [2] with modifications as described in detail by Hermund et al. [94] and quantified through gas chromatography with flame-ionization detection (GC-FID). Gas chromatography was performed on an Agilent HP 5890A (Agilent Technologies, Palo Alto, California, USA) with a DB WAX 127-7012 column (10 m x 0.1 mm x 0.1 µm film thickness, Agilent Technologies). The injection volume was 0.2 µL in split mode (1:50) and He was used as carrier gas. A predefined temperature program was used (160 ℃ - 200 ℃ at a rate of 10.6 ℃ min^{-1}, 200 ℃ for 0.3 min, 200 ℃ - 220 ℃ at a rate of 10.6 ℃ min^{-1}, 220 ℃ for 1 min, 220 ℃ - 240 ℃ at a rate of 10.6 ℃ min^{-1} and 240 ℃ for 3.8 min). The fatty acids were identified by comparison with the fatty acid standard Nu-Check-prep GLC 714 (Nu-Check Prep, Inc., Elysian, Minnesota, USA).

Amino acids and protein

Amino acids were determined in duplicate by acid hydrolysis, derivatized using the EZfaast kit (Phenomenex Ltd. Deutschland, Aschaffenburg, Germany) and quantified through LC-MS. Seaweed powder was hydrolysed in 1 mL 6M HCl, further diluted and filtered prior to analysis

on the LC-MS and quantification against known standards. This method completely degrades cysteine (CYS) and tryptophan (TRP), while asparagine (ASN) is converted to aspartic acid (ASP). We therefore reported the results for cystine (CC) and aspartic acid (ASP), which represents the sum of asparagine and aspartic acid, along with the other amino acids.

Protein content was calculated by summarising the total weight of proteinogenic amino acid residues (the anhydrous fraction of the amino acid found in the polypeptide chain) per sample. For this purpose, hydroxyproline (HYP) was considered as non-proteinogenic. The results are expressed as mg kg^{-1} dry weight (dw).

Essential amino acid (EAA) profiles were compared with the EAA requirement for 3 - 14 years school child/adolescent. The resulting amino acid score (AAS) was calculated by the 2007 method of WHO/FAO/UNO, but using the reference protein pattern for 3-10 year olds as recommended in Food and Agriculture Organization of the United Nations [77]:

$$\text{Amino acid score (AAS, \%)} = \frac{\text{mg limiting amino acid per g of test protein}}{\text{mg limiting amino acid per g of reference protein pattern}} * 100 \tag{6.1}$$

Antioxidant activity

Methanol extracts An aliquot of 200 mg freeze dried powder was weighed into a 10 mL centrifuge tube. Five mL methanol was added, and the tubes sonicated for 15 min. Then they were centrifuged for 10 min at 5311 × g and the solvent layer separated. The methanol addition and subsequent treatment was repeated, and the solvent layers combined. Methanol was evaporated and the sample redissolved at 5 mg extract per mL methanol. We used methanol as a solvent in this study because the aim of the extraction of antioxidants was to obtain as much antioxidant as possible to be able to assess the total content of phenolic compounds. The aim was not to obtain an extract, which could be used for addition to food products.

Total phenolic content (TPC) Total phenolic content was determined spectrophotometrically by using the Folin–Ciocâlteu reagent as described in detail by Farvin and Jacobsen [76] with the modifications by Hermund et al. [94]. Briefly, methanol extracts in different concentrations were incubated with the Folin–Ciocâlteu reagent and measured on a UV–VIS spectrophotometer (Shimadzu UV mini 1240, Duisburg, Germany). The results were quantified against a standard curve prepared from serially diluted gallic acid (Sigma-Aldrich, St. Louis, Missouri, USA). The results are expressed as µg gallic acid equivalents (GAE) g^{-1} dry weight (dw).

(1,1-diphenyl-2-picrylhydrazyl) (DPPH) method The scavenging effect on the free radical from 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined spectrophotometrically as described by Yang, Guo, and Yuan [246], modified as described by Hermund et al. [94], and adapted for measurement in a multiplate reader. Briefly, methanol extracts were incubated

with DPPH at different concentrations and measured on a UV–VIS spectrophotometer in triplicate. Blanks and a control (butylated hydroxytoluene, Sigma-Aldrich, St. Louis, Missouri, USA) were determined alongside the samples. DPPH radical scavenging capacity was determined with the equation

$$\text{DPPH radical scavenging capacity (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{blank}}}\right) \times 100, \qquad (6.2)$$

where A is the absorbance. The concentration of antioxidant necessary to inhibit 50 % of DPPH, EC_{50}, was calculated by linear regression of the dose-response curve and is reported as mg extract mL^{-1} reagent.

Statistical analyses

Data analysis and visualisation was carried out with R version 3.4.4 (2018-03-15) [181] in RStudio version 1.1.463 [193] on a x86_64-pc-linux-gnu (64-bit) platform, using the following packages: readxl [242], dplyr [243], stats [181], utils [181], and ggplot2 [241].

Normality was assessed with the Shapiro-Wilk test and homogeneity with the Bartlett Test of Homogeneity of Variances. Where preconditions for parametric tests were not met, the Kruskal-Wallis test was used to compare differences between species. Principal component analysis (PCA) was carried out using the prcomp function (stats package 3.4.4) with centring and subsequent scaling of samples by their standard deviation. A confidence level of 95 % was used unless otherwise noted.

6.4 Results and discussion

6.4.1 Dry matter and ash

The dry matter content ranged from 12.0 ± 2.6 to 24.5 ± 4.5 g per 100 g wet weight (median concentration ± median absolute deviation, based on the wet weight of the initial sample before freeze drying), see table 6.1. Fucoids (*A. nodosum*, *F. distichus* and *F. vesiculosus*) had the highest dry matter content of more than 20.9 g per 100 g wet weight, which was significantly higher than the dry matter content of *A. esculenta*, which had the lowest median dry matter content. The high content of dry matter in the fucoids may be part of drought-tolerance strategies evolved to cope with growing in the littoral zone [201]. Schonbeck and Norton [201] found a positive correlation between dry matter content and drought-tolerance in fucoid species.

Ash content was highest in *A. esculenta* (31.5 ± 5.2 g (100 g dry weight (dw))$^{-1}$) and lowest in *A. clathratum* (20.5 ± 4.0 g (100 g dw)$^{-1}$) with no evident patterns. About half of the ash content was made up of 16 elements (As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Se and Zn) investigated in our previous study [114], listed as 'minerals' in table 6.1. The findings for dry matter and ash contents of this study are comparable to what was reported for *A. nodosum*, *Fucus*, *Laminaria* and *Saccharina* species as well as *P. palmata* in a review by Holdt

Table 6.1: Median content ± median absolute deviation of dry matter and ash content for seaweeds from Greenland. 'Mineral' contents are reported based on Kreissig et al. [114]. Median content ± median absolute deviation for dry matter and ash are reported as g (100 g dry weight)$^{-1}$ and 'minerals' as g (100 g freeze dried weight)$^{-1}$.

Species	n	Dry matter	Ash	'Minerals'*
Agarum clathratum	3	17.3 ± 0.4	20.5 ± 4.0	10.4 ± 3.1
Alaria esculenta	8	12.0 ± 2.6	31.5 ± 5.2	15.7 ± 4.9
Ascophyllum nodosum	6	24.5 ± 4.8	22.2 ± 1.0	8.3 ± 0.9
Fucus distichus	5	20.9 ± 4.4	23.2 ± 2.1	10.3 ± 1.0
Fucus vesiculosus	9	23.6 ± 3.3	22.1 ± 1.6	8.9 ± 1.3
Hedophyllum nigripes	4	16.7 ± 6.3	26.9 ± 7.0	14.5 ± 3.9
Laminaria solidungula	5	15.3 ± 3.3	23.6 ± 2.2	13.6 ± 3.6
Palmaria palmata	2	14.4 ± 4.3	23.0 ± 0.1	12.2 ± 3.6
Saccharina latissima	6	16.3 ± 1.1	22.3 ± 2.8	11.6 ± 3.4
Saccharina longicruris	2	16.1 ± 4.4	31.0 ± 8.4	16.8 ± 6.2

* Based on the sum of As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Se and Zn.

and Kraan [98]. Mæhre et al. [136] reported dry matter (as (100 g dw)$^{-1}$) and ash contents (as (100 g freeze dried weight)$^{-1}$) for *A. esculenta* at 17.4 ± 0.5 and 24.6 ± 0.6, *F. vesiculosus* at 34.3 ± 2.1 and 20.9 ± 0.1 and *P. palmata* at 18.1 ± 0.6 and 28.8 ± 0.2 for seaweed collected in Norway. Compared to the study by Mæhre et al. [136], the results from the present study are lower in dry matter content for the two brown seaweed species, and slightly higher in ash content for all three species. However, since Mæhre et al. [136] only analysed three samples per species, and reported ash content per freeze dried weight, which tends to retain a small degree of moisture, these differences are not significant. Dry matter content of *L. solidungula* was in the range (9 to 18 %) reported by Chapman and Lindley [33] for Canadian *L. solidungula*. We could not find any published literature on ash content in *L. solidungula*, or dry matter and ash content in both *A. clathratum* and *H. nigripes*.

6.4.2 Fatty acids

The median content (± median absolute deviation) of fatty acids ranged between 5 130 ± 1 370 mg kg^{-1} dw for *H. nigripes* and 37 500 ± 7 870 mg kg^{-1} dw for *F. vesiculosus* with notable differences in the fatty acid profiles (see table 6.2, page 57 and table 6.3, page 58). The total content of n-3 fatty acids ranged between 659 ± 214 mg kg^{-1} dw for *H. nigripes* and 4 060 ± 761 mg kg^{-1} dw for *F. vesiculosus*. The total content of n-6 fatty acids ranged between 515 ± 150 mg kg^{-1} dw for *P. palmata* and 11 110 ± 1 310 mg kg^{-1} dw for *F. vesiculosus*.

Table 6.2: Fatty acid composition of the seaweeds *A. clathratum*, *A. esculenta*, *A. nodosum*, *F. distichus* and *F. vesiculosus* from Greenland. Median content ± median absolute deviation in mg kg^{-1} dry weight.

Fatty acid	*A. clathratum*	*A. esculenta*	*A. nodosum*	*F. distichus*	*F. vesiculosus*
14:0	705 ± 710	472 ± 89	2040 ± 575	2910 ± 117	3920 ± 492
14:1	18.1 ± 1.0	6.14 ± 6.75	36.6 ± 45.5	38.5 ± 19.3	39.8 ± 9.7
15:0	73 ± 10	28.9 ± 15.6	50.9 ± 34.0	115 ± 24	85 ± 43
16:0	3300 ± 3080	1540 ± 336	2320 ± 504	4620 ± 776	3980 ± 598
16:1 (n-7)	1520 ± 168	546 ± 58	431 ± 136	623 ± 167	621 ± 54
16:2 (n-4)	219 ± 162	14.4 ± 7.6	62.2 ± 22.8	66.3 ± 6.6	42.7 ± 11.4
16:3 (n-4)	134 ± 3	17.5 ± 8.0	46.7 ± 36.3	107 ± 8	88.2 ± 16.4
17:0	127 ± 81	13.8 ± 6.3	29.3 ± 6.2	48.5 ± 10.7	59.2 ± 13.9
16:4 (n-3)	564 ± 309	24.7 ± 7.7	8.82 ± 4.74	16.4 ± 8.3	25 ± 23
18:0	315 ± 376	90.8 ± 26.9	77.4 ± 66.0	164 ± 45	87.1 ± 50.6
18:1 (n-9)	2130 ± 2760	807 ± 109	8620 ± 4840	7030 ± 715	12700 ± 4400
18:1 (n-7)	87.0 ± 61.5	62.1 ± 53.3	11.9 ± 3.7	13.8 ± 15.7	25.6 ± 2.3
18:2 (n-6)	1320 ± 1790	572 ± 162	2110 ± 1120	4560 ± 234	4800 ± 712
18:2 (n-4)	77.9 ± 98.4	41.3 ± 23.8	96.2 ± 13.0	172 ± 62	152 ± 21
18:3 (n-6)	823 ± 986	688 ± 254	969 ± 255	1630 ± 184	1960 ± 119
18:3 (n-4)	154 ± 49	0 ± 0	4.39 ± 5.66	0 ± 0	0 ± 0
18:3 (n-3)	377 ± 408	1020 ± 209	751 ± 107	986 ± 194	1100 ± 228
18:4 (n-3)	66 ± 20	3.04 ± 1.87	15.2 ± 9.0	5.79 ± 7.45	13.1 ± 1.0
20:0	132 ± 146	53 ± 22	34.1 ± 11.9	71.3 ± 10.1	109 ± 17
20:1 (n-9, n-11)	34.9 ± 15.0	20.9 ± 14.1	117 ± 41	138 ± 31	335 ± 57
20:1 (n-7)	3.66 ± 5.43	4.89 ± 7.24	0 ± 0	0 ± 0	0 ± 0
20:2 (n-6)	22.5 ± 2.4	18.9 ± 4.9	370 ± 291	110 ± 23	229 ± 36
20:3 (n-6)	91.5 ± 123.0	22.5 ± 6.1	296 ± 69	467 ± 48	636 ± 138
20:4 (n-6)	849 ± 858	607 ± 168	2890 ± 1070	2590 ± 161	3490 ± 303
20:3 (n-3)	14.7 ± 11.0	9.36 ± 6.31	115 ± 52	28.1 ± 8.2	73.4 ± 10.6
20:4 (n-3)	81.7 ± 92.3	110 ± 49	112 ± 52	216 ± 19	248 ± 41
20:5 (n-3)	1380 ± 1710	1290 ± 580	1530 ± 471	1740 ± 118	2220 ± 388
22:1 (n-11)	13.2 ± 1.7	12.8 ± 7.1	11.9 ± 3.8	13.3 ± 3.2	14.3 ± 2.6
22:1 (n-9)	8.11 ± 11.50	5.97 ± 6.92	0 ± 0	0 ± 0	0 ± 0
21:5 (n-3)	1.20 ± 1.77	4.71 ± 3.13	7.78 ± 9.53	1.39 ± 2.06	6.33 ± 3.30
22:5 (n-3)	786 ± 776	8.89 ± 3.51	8.34 ± 8.63	15.4 ± 8.3	14.8 ± 10.8
22:6 (n-3)	41.2 ± 1.1	28.2 ± 21.2	300 ± 72	371 ± 18	363 ± 55
24:1 (n-9)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
22:0	62.6 ± 43.5	24.5 ± 10.6	32.2 ± 12.1	43.4 ± 14.1	44.7 ± 13.8
Σn-3	3310 ± 3330	2500 ± 881	2850 ± 786	3380 ± 383	4060 ± 761
Σn-6	3100 ± 3760	1910 ± 594	6640 ± 2800	9360 ± 649	11100 ± 1310
n-6/n-3	0.938 ± 1.130	0.764 ± 0.674	2.33 ± 3.57	2.77 ± 1.70	2.74 ± 1.72
EFA*	1690 ± 2200	1590 ± 371	2860 ± 1230	5540 ± 428	5900 ± 939

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Fatty acid composition of the seaweeds *A. clathratum*, *A. esculenta*, *A. nodosum*, *F. distichus* and *F. vesiculosus* from Greenland. Median content ± median absolute deviation in mg kg^{-1} dry weight. (Continued from previous page)

Fatty acid	*A. clathratum*	*A. esculenta*	*A. nodosum*	*F. distichus*	*F. vesiculosus*
ΣFA	15500 ± 14900	8170 ± 2280	23500 ± 9940	28900 ± 3060	37500 ± 7870

* Essential fatty acis (EFA): 18:3 (n-3) + 18:2 (n-6)

Table 6.3: Fatty acid composition of the seaweeds *H. nigripes*, *L. solidungula*, *P. palmata*, *S. latissima* and *S. longicruris* from Greenland. Median content ± median absolute deviation in mg kg^{-1} dry weight.

Fatty acid	*H. nigripes*	*L. solidungula*	*P. palmata*	*S. latissima*	*S. longicruris*
14:0	539 ± 144	387 ± 56	700 ± 245	1130 ± 130	591 ± 286
14:1	11.3 ± 8.5	0 ± 0	0 ± 0	9.87 ± 12.90	3.21 ± 4.76
15:0	14.1 ± 14.5	18.9 ± 19.8	29.8 ± 13.2	48.2 ± 14.4	12.0 ± 17.7
16:0	1290 ± 244	1050 ± 154	2310 ± 425	2600 ± 682	1200 ± 257
16:1 (n-7)	588 ± 83	312 ± 24	384 ± 31	462 ± 186	512 ± 124
16:2 (n-4)	27.5 ± 22.9	17.0 ± 6.5	163 ± 233	49.3 ± 8.3	70.8 ± 60.4
16:3 (n-4)	6.85 ± 2.89	30.8 ± 8.0	3.72 ± 1.46	28.6 ± 11.1	6.55 ± 1.06
17:0	9.20 ± 1.00	9.40 ± 4.65	32.5 ± 22.8	28.8 ± 16.1	7.80 ± 3.36
16:4 (n-3)	15.9 ± 5.7	12.5 ± 9.5	12.1 ± 7.3	27.0 ± 19.1	16.8 ± 7.0
18:0	29.6 ± 8.8	24.5 ± 3.1	63.2 ± 2.5	156 ± 84	46.0 ± 4.8
18:1 (n-9)	705 ± 338	454 ± 82	380 ± 134	3390 ± 2390	704 ± 197
18:1 (n-7)	19.6 ± 14.8	24.5 ± 15.4	208 ± 33	3.06 ± 2.78	34.6 ± 8.9
18:2 (n-6)	350 ± 126	544 ± 78	231 ± 85	1980 ± 977	353 ± 23
18:2 (n-4)	38.7 ± 4.0	18.4 ± 8.2	21.0 ± 7.3	134 ± 70	30.9 ± 6.2
18:3 (n-6)	180 ± 49	407 ± 101	152 ± 9	981 ± 707	230 ± 1
18:3 (n-4)	4.00 ± 3.92	0 ± 0	0 ± 0	1.20 ± 1.78	203 ± 290
18:3 (n-3)	282 ± 151	470 ± 145	168 ± 120	600 ± 386	165 ± 242
18:4 (n-3)	4.13 ± 4.79	0 ± 0	3.27 ± 0.09	8.21 ± 4.24	3.37 ± 1.62
20:0	24.6 ± 9.9	19.3 ± 6.5	9.23 ± 5.34	88.6 ± 29.5	18.2 ± 3.4
20:1 (n-9, n-11)	13.1 ± 8.1	12.3 ± 3.9	51.7 ± 26.6	36.9 ± 18.4	13.9 ± 20.6
20:1 (n-7)	0 ± 0	4.05 ± 6.00	3.43 ± 5.08	0 ± 0	5.34 ± 0.53
20:2 (n-6)	8.76 ± 0.37	21.6 ± 4.2	14.2 ± 12.3	16.5 ± 2.6	9.28 ± 0.39
20:3 (n-6)	27.9 ± 15.2	17.2 ± 3.9	12.1 ± 1.3	161 ± 114	23.6 ± 0.3
20:4 (n-6)	538 ± 49	488 ± 104	105 ± 43	1150 ± 574	421 ± 40
20:3 (n-3)	7.10 ± 1.31	12.1 ± 2.1	6.46 ± 2.76	8.95 ± 2.90	4.61 ± 1.19
20:4 (n-3)	32.7 ± 16.3	52.3 ± 21.6	22.3 ± 2.8	151 ± 156	41.0 ± 4.8
20:5 (n-3)	283 ± 26	768 ± 137	3360 ± 585	1250 ± 961	492 ± 38
22:1 (n-11)	4.43 ± 1.33	7.29 ± 4.77	42.8 ± 12.8	8.23 ± 5.20	6.69 ± 0.58
22:1 (n-9)	2.88 ± 2.87	0 ± 0	3.66 ± 2.39	0 ± 0	7.69 ± 5.02

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Fatty acid composition of the seaweeds *H. nigripes*, *L. solidungula*, *P. palmata*, *S. latissima* and *S. longicruris* from Greenland. Median content ± median absolute deviation in mg kg^{-1} dry weight. (Continued from previous page)

Fatty acid	*H. nigripes*	*L. solidungula*	*P. palmata*	*S. latissima*	*S. longicruris*
21:5 (n-3)	0 ± 0	2.72 ± 4.04	0 ± 0	7.46 ± 2.33	10.5 ± 13.7
22:5 (n-3)	14.1 ± 4.2	19.1 ± 19.0	10.6 ± 0.9	22.7 ± 10.3	10.1 ± 5.1
22:6 (n-3)	19.3 ± 5.5	19.4 ± 8.2	140 ± 47	14.8 ± 4.0	24.3 ± 25.3
24:1 (n-9)	0.569 ± 0.844	0 ± 0	0 ± 0	2.80 ± 4.15	3.41 ± 5.05
22:0	41.0 ± 5.1	55.3 ± 17.5	27.1 ± 1.7	47.4 ± 15.5	20.7 ± 8.1
Σn-3	659 ± 214	1360 ± 347	3720 ± 766	2090 ± 1550	767 ± 338
Σn-6	1100 ± 239	1480 ± 290	515 ± 150	4280 ± 2370	1040 ± 65
n-6/n-3	1.68 ± 1.11	1.09 ± 0.84	0.138 ± 0.196	2.05 ± 1.54	1.35 ± 0.19
EFA*	632 ± 277	1010 ± 223	400 ± 205	2580 ± 1360	517 ± 265
ΣFA	5130 ± 1370	5270 ± 1060	8670 ± 2120	14600 ± 7600	5300 ± 1710

* Essential fatty acis (EFA): 18:3 (n-3) + 18:2 (n-6)

A dietary n-6/n-3 ratio of 1 to 2 is recommended for modern diets to promote health as well as to prevent and manage obesity [209]. In addition to contributing negatively to atherosclerosis, obesity, and diabetes, a high n-6/n-3 ratio has also been associated with an increased risk for mood disorders in young people in high-risk groups [19]. The determined seaweed n-6/n-3 ratios ranged from 0.14 ± 0.20 for *P. palmata* to 2.77 ± 1.70 for *F. distichus*. However, the other two fucoids investigated (*A. nodosum* and *F. vesiculosus*) also had ratios over 2.3. Since many modern Western diets are characterised by high ratios of n-6/n-3, the inclusion of *P. palmata* could help improve the health impact of these diets. *Alaria esculenta* had a n-6/n-3 ratio of 0.81 which is close to the palaeolithic[1] diet n-6/n-3 ratio of 0.79 that is theorized by Simopoulos [209] to be the ideal ratio for human diets.

The content of linoleic acid 18:2 (n-6) was highest in *F. vesiculosus* (4 800 ± 712 mg kg^{-1} dw), and lowest in *P. palmata* (231 ± 85 mg kg^{-1} dw). *Fucus vesiculosus* also had the highest content of α-linolenic acid 18:3 (n–3) at 1 100 ± 228 mg kg^{-1} dw, while *S. longicruris* had the lowest content (165 ± 242 mg kg^{-1} dw). The median content of eicosapentaenoic acid (EPA) 20:5(n-3) was highest in *P. palmata* (3 360 ± 585 g kg^{-1} dw) and lowest in *H. nigripes* (283 ± 26 g kg^{-1} dw). The content of arachidonic acid 20:4 (n-6) was highest in *F. vesiculosus* (3 490 ± 303 mg kg^{-1} dw), and lowest in *P. palmata* (105 ± 43 mg kg^{-1} dw). The content of docosahexaenoic acid (DHA) 22:6 (n-3) was highest in *F. distichus* (371 ± 18 mg kg^{-1} dw) and lowest in *S. latissima* (14.8 ± 4.0 mg kg^{-1} dw).

Agarum clathratum had a high content of docosapentaenoic acid (DPA) 22:5 (n-3), with 262 to 1 401 mg kg^{-1} dw compared to the other species in this study, which were all below 23 mg

[1] "Paleolithic Period, also spelled Palaeolithic Period, also called Old Stone Age, ancient cultural stage, or level, of human development, characterized by the use of rudimentary chipped stone tools." Definition from Britannica.com [63]

kg^{-1} dw. There is increasing evidence that DPA may have shared biological effects with EPA and DHA, in addition to specific beneficial effects when consumed by humans, such as reducing risk of cardiovascular disease [51].

The results from the present study were compared with relevant published literature on material of Arctic or Nordic origin. Generally, we observed the same patterns for the selected important fatty acids (α-linolenic acid 18:3 (n–3), linoleic acid 18:2 (n-6), arachidonic acid 20:4 (n-6), EPA 20:5 (n-3), DHA 22:6 (n–3), as well as the sum of n-3 and n-6 acids and n-6/n-3 ratio) as those reported in other studies. However, we could not find any published studies on *H. nigripes*. The fatty acid pattern from the present study fell within the range observed by Graeve et al. [85] for *L. solidungula* and *P. palmata* collected in Spitsbergen (Kongsfjorden, Ny-Ålesund, Spitsbergen, Arctic, 78°56' N, 11° 56' E) and Mæhre et al. [136] for *A. esculenta, F. vesiculosus* and *P. palmata* collected in Norway, but much further South (Voldsfjorden, Møre and Romsdal county (62° N, 5° E)). The profile observed for *L. solidungula* in this study is mostly in accordance with findings for mid to old fronds reported by Graeve et al. [85]. It should be noted that in the present study, there was no differentiation between the age of the frond parts. For *S. latissima*, our results from samples collected between June and September were comparable to Irish *S. latissima* collected in June by Schmid, Guihéneuf, and Stengel [199] .

Multivariate statistical analysis of the fatty acid profiles using principal component analysis (PCA) revealed that 60 % of the variance in the data could be explained by the first two principal components. Figure 6.1 (page 61) shows some overlap between fucoids and larger kelp species (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*). The apparent clustering of fucoids in three distinct groups was not related to sampling location. Monteiro et al. [146] have recently shown that fatty acid profiles can be used to distinguish *S. latissima* from different geographic locations (France, Norway, and UK). Thus, a more thorough investigation into geographical differences for Greenland seaweed could perhaps reveal a similar result.

The loadings plot shown in figure 6.2 (page 62) illustrates that kelp species and *P. palmata* were characterised by a higher amount of 18:1 (n-7), 20:1 (n-7) and 22:1 (n-9), which all lie in the negative part of the PC1 axis and the positive part of the PC2 axis, while fucoids were characterised by higher concentrations of EPA and DHA (marked in blue in figure 6.2, page 62), and n-6 fatty acids. *Agarum clathratum* was characterised by a uniquely high concentration of 16:4 (n-3) and 22:5 (n-3) (see table 6.2, page 57), which is reflected in 6.2 (page 62): *Agarum clathratum* contains at least 20 times as much 16:4 (n-3) as the other investigated species, and 35 times as much 22:5 (n-3). Kelly et al. [109] also found higher concentrations of 22:5 (n-3) in *A. clathratum* compared to, amongst others, *S. latissima*.

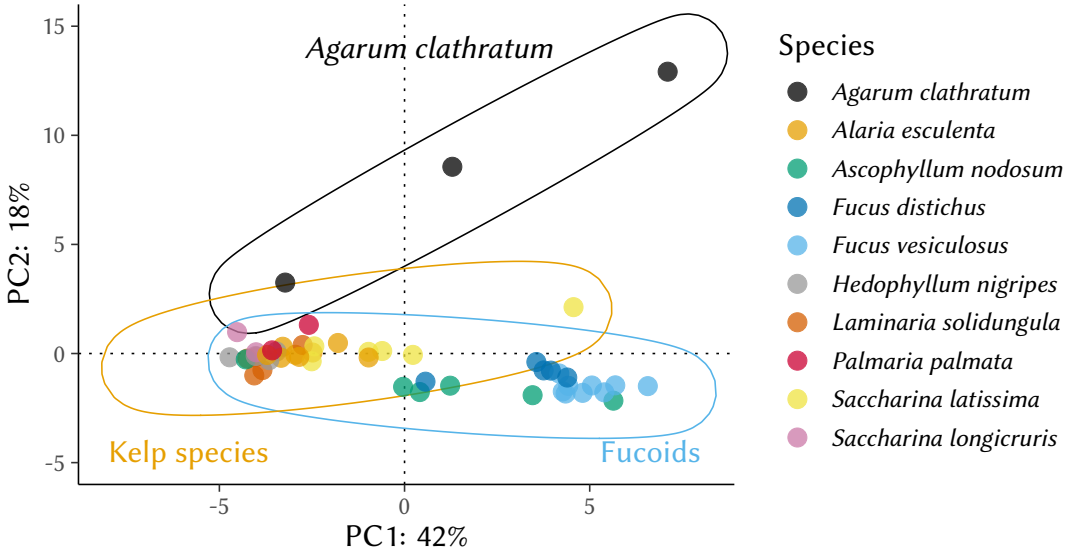

Figure 6.1: Principal component analysis of fatty acid composition in seaweeds from Greenland. Ellipses mark the kelp species (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*), with *A. clathratum* marked separately, and fucoids (*A. nodosum*, *F. distichus* and *F. vesiculosus*). *Palmaria palmata* (in pink), the only red macroalga investigated, lies within the ellipse marking the kelp species.

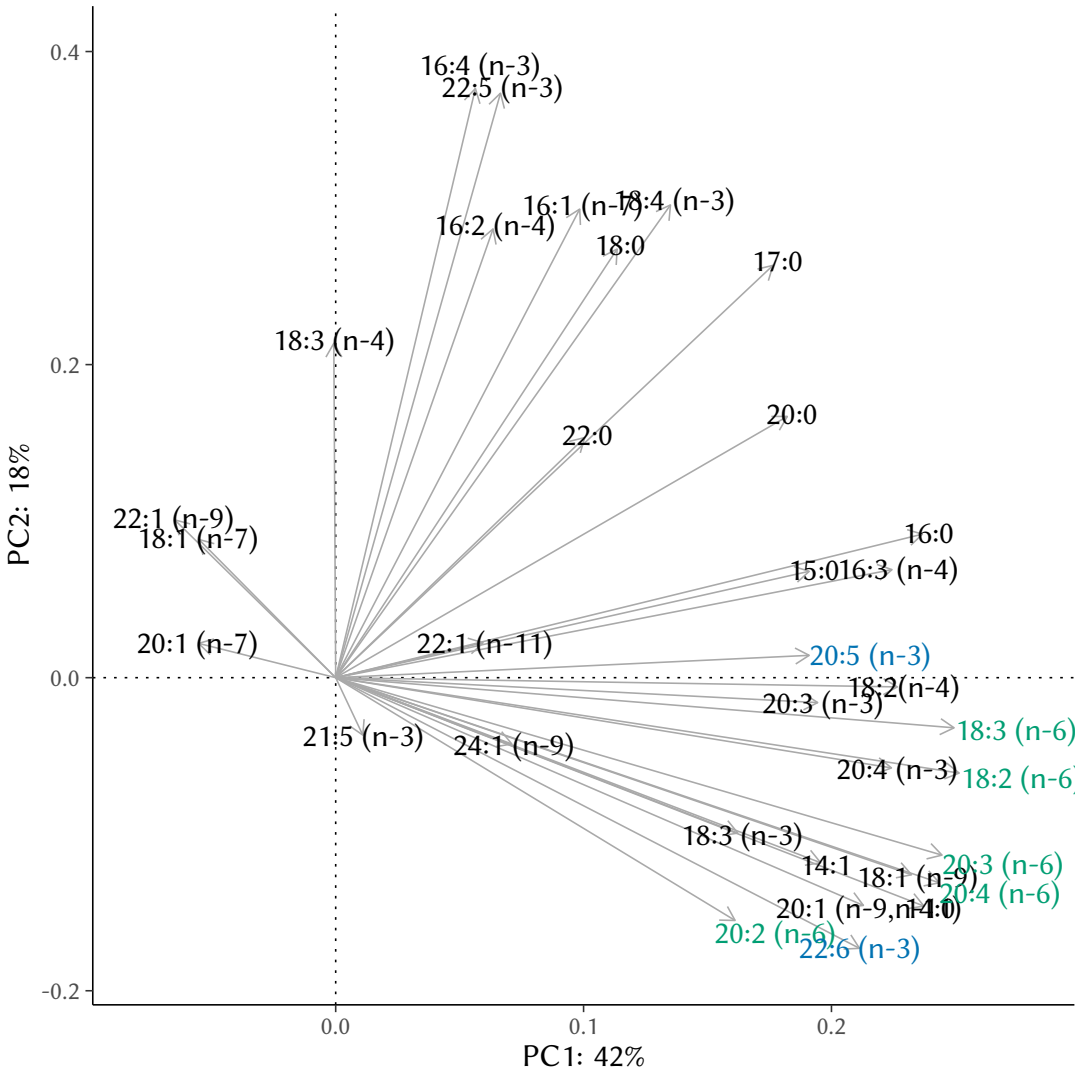

Figure 6.2: Principal component analysis loadings plot of fatty acid composition in seaweeds from Greenland. Eicosapentaenoic acid (EPA) 20:5 (n-3) and docosahexaenoic acid (DHA) 22:6 (n-3) are both marked in blue, n-6 fatty acids are marked in green.

6.4.3 Amino acids

The protein content ranged from 4.6 ± 1.7 % dw for *L. solidungula* to 7.1 ± 1.7 % dw for *A. clathratum* for the brown seaweeds, with much higher concentrations being observed for the red seaweed *P. palmata* at 12 ± 3 % dw (see table 6.4, page 63 and table 6.5, page 64), which is comparable to studies on *P. palmata* summarised in Holdt and Kraan [98]. The protein content for *S. latissima* of 5.2 ± 2.8% found in this study lies within the ranges reported by Bak [10] for Faroese *S. latissima* with 4.3 ± 0.9 % dw and Marinho et al. [139] for Danish *S. latissima* with 1.3 to 10.8 % dw.

Table 6.4: Amino acid composition of the seaweeds *A. clathratum*, *A. esculenta*, *A. nodosum*, *F. distichus* and *F. vesiculosus* (mg g^{-1} dry weight) from Greenland.

Amino acid	*A. clathratum*	*A. esculenta*	*A. nodosum*	*F. distichus*	*F. vesiculosus*
ALA	5.28 ± 1.46	6.84 ± 2.73	3.69 ± 0.63	3.63 ± 0.54	3.97 ± 1.24
ARG	2.36 ± 0.52	2.65 ± 1.10	1.72 ± 0.14	1.88 ± 0.70	2.09 ± 0.39
ASP	11.3 ± 2.6	9.60 ± 4.35	9.90 ± 0.48	11.0 ± 0.4	10.2 ± 2.7
CC	0.90 ± 0.25	0.06 ± 0.08	0.06 ± 0.03	0.06 ± 0.05	0.08 ± 0.04
GLU	11.4 ± 4.8	9.84 ± 3.10	13.9 ± 4.4	10.8 ± 3.9	10.4 ± 4.7
GLY	4.23 ± 0.85	3.98 ± 1.60	2.59 ± 0.19	2.74 ± 0.16	3.12 ± 0.71
HIS	0.87 ± 0.50	1.29 ± 1.05	1.02 ± 0.16	0.82 ± 0.33	1.12 ± 0.62
HYP	0.06 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ILE	4.14 ± 0.98	4.01 ± 1.60	2.39 ± 0.25	3.47 ± 0.77	3.00 ± 0.31
LEU	5.84 ± 1.28	5.98 ± 2.54	3.68 ± 0.15	4.51 ± 0.43	4.37 ± 0.82
LYS	6.74 ± 1.74	4.84 ± 2.48	3.68 ± 0.41	4.64 ± 1.13	4.59 ± 0.83
MET	0.62 ± 0.06	1.20 ± 0.84	1.17 ± 0.03	1.07 ± 0.68	1.19 ± 0.31
PHE	3.83 ± 0.47	3.46 ± 1.16	2.31 ± 0.10	2.26 ± 0.22	2.53 ± 0.28
PRO	5.05 ± 1.06	3.13 ± 1.27	2.15 ± 0.12	2.20 ± 0.32	2.33 ± 0.43
SER	4.40 ± 1.95	3.40 ± 1.52	2.42 ± 0.21	2.46 ± 0.60	2.99 ± 0.71
THR	3.83 ± 0.22	3.79 ± 1.54	2.54 ± 0.20	2.71 ± 0.36	2.90 ± 0.44
TYR	2.01 ± 0.40	1.99 ± 0.56	1.07 ± 0.06	1.37 ± 0.35	1.28 ± 0.34
VAL	4.56 ± 0.53	4.80 ± 2.29	3.26 ± 0.36	3.49 ± 0.50	3.60 ± 0.83
ΣEAA*	30.4 ± 6.0	29.7 ± 13.8	19.9 ± 0.5	22.1 ± 3.3	23.2 ± 3.5
ΣAA	72.0 ± 6.3	69.1 ± 29.2	57.4 ± 5.8	62.2 ± 4.0	56.9 ± 10.7
EAA/ΣAA	0.39 ± 0.05	0.43 ± 0.03	0.34 ± 0.02	0.40 ± 0.02	0.39 ± 0.02
Protein	61.5 ± 5.2	59.0 ± 24.9	49.3 ± 5.0	53.4 ± 3.4	48.6 ± 9.1
AAscore	96	127	131	122	142

* Essential amino acids (EAA): histidine (HIS), isoleucine (ISO), leucine (LEU), lysine (LYS), methionine (MET), phenylalanine (PHE), threonine (THR), tryptophan (TRP) and valine (VAL)

Table 6.5: Amino acid composition of the seaweeds *H. nigripes*, *L. solidungula*, *P. palmata*, *S. latissima* and *S. longicruris* (mg g^{-1} dry weight) from Greenland.

Amino acid	*H. nigripes*	*L. solidungula*	*P. palmata*	*S. latissima*	*S. longicruris*
ALA	10.6 ± 5.0	5.13 ± 2.58	11.50 ± 1.0	6.33 ± 5.04	6.34 ± 1.68
ARG	2.38 ± 0.64	1.34 ± 0.35	6.70 ± 1.57	1.95 ± 0.71	2.55 ± 0.25
ASP	11.6 ± 2.1	6.86 ± 2.80	18.8 ± 6.5	6.98 ± 4.07	12.8 ± 4.2
CC	0.09 ± 0.08	0.07 ± 0.02	0.65 ± 0.12	0.04 ± 0.04	0.01 ± 0.02
GLU	11.9 ± 4.9	5.42 ± 3.30	18.2 ± 8.4	8.70 ± 4.89	9.77 ± 3.69
GLY	3.94 ± 0.79	3.39 ± 1.07	8.79 ± 1.99	3.30 ± 1.85	3.78 ± 0.61
HIS	1.27 ± 0.36	0.56 ± 0.07	2.53 ± 0.53	0.68 ± 0.59	1.49 ± 0.27
HYP	0.22 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.06	0.10 ± 0.02
ILE	3.52 ± 0.32	2.52 ± 0.65	6.72 ± 2.31	3.03 ± 1.26	2.66 ± 0.84
LEU	5.40 ± 0.14	4.37 ± 1.18	10.1 ± 1.9	4.86 ± 1.70	4.78 ± 0.40
LYS	6.24 ± 1.71	3.88 ± 2.34	11.2 ± 0.4	4.89 ± 2.61	4.86 ± 1.73
MET	1.28 ± 0.68	0.86 ± 0.25	2.37 ± 0.23	1.30 ± 0.51	1.35 ± 0.11
PHE	3.82 ± 0.15	2.34 ± 0.94	5.95 ± 1.23	2.73 ± 1.44	3.13 ± 0.41
PRO	3.48 ± 0.45	3.01 ± 0.62	10.2 ± 5.1	3.11 ± 2.12	3.22 ± 0.79
SER	4.13 ± 0.86	3.27 ± 1.60	7.52 ± 1.59	2.54 ± 2.12	3.75 ± 0.47
THR	5.02 ± 0.12	3.48 ± 1.28	6.71 ± 1.44	2.99 ± 1.95	3.58 ± 0.40
TYR	2.08 ± 0.36	1.78 ± 0.45	3.80 ± 0.81	1.46 ± 0.37	1.79 ± 0.31
VAL	4.69 ± 1.05	3.53 ± 0.85	9.76 ± 2.12	3.99 ± 1.80	4.33 ± 1.31
ΣEAA*	30.4 ± 3.1	21.7 ± 6.7	55.4 ± 10.2	24.6 ± 12.9	26.2 ± 5.5
ΣAA	83.9 ± 20.9	54.6 ± 19.6	142 ± 37	61.4 ± 33.9	70.3 ± 17.5
ΣEAA/ΣAA	0.38 ± 0.04	0.42 ± 0.02	0.40 ± 0.03	0.42 ± 0.05	0.37 ± 0.02
Protein	71.1 ± 17.5	46.4 ± 16.6	121 ± 32	52.3 ± 28.8	60.0 ± 15.0
AAscore	117	89	131	102	155

* Essential amino acids (EAA): histidine (HIS), isoleucine (ISO), leucine (LEU), lysine (LYS), methionine (MET), phenylalanine (PHE), threonine (THR), tryptophan (TRP) and valine (VAL)

The dominating amino acids were aspartic acid (ASP) and glutamic acid (GLU) in all investigated seaweeds. The ratio of essential amino acids (EAA: phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine) to total amino acids (EAA/AA), a quality indicator for a studied protein source, was between 0.35 and 0.41. For the red seaweed *P. palmata*, the EAA/AA ratio was 0.40, slightly lower than what has been reported for red seaweed by Naseri, Holdt, and Jacobsen [156]. It should be noted here that the essential amino acid tryptophan was not quantified in this study, nor by Naseri, Holdt, and Jacobsen [156], leading to lower EAA content than if tryptophan had been quantified. Bak [10] found EAA/AA ratios of 0.32 to 0.52 for Faroese *S. latissima*, so the ratio of 0.42 for *S. latissima*

found in this study lies within this range. Comparing EAA/AA ratios from Lorenzo et al. [133] and our study showed similar ratios for *A. nodosum* (0.39 and 0.34) and *F. vesiculosus* (0.41 and 0.39) respectively.

The amino acid scores were high, between 89 % for *L. solidungula* and 155 % for *S. longicruris*, comparable to what Anagnostara [3] found for dried *S. latissima*. No pattern was discernible between fucoids and kelp species. The limiting amino acid was histidine for all species. This evaluation is with reservations, since we did not quantify cysteine nor tryptophan, both of which are included in the scoring pattern. Studies on seaweed which include cysteine and tryptophan are rare, since most studies employ methods that degrade these amino acids. Bocanegra et al. [22] list *P. palmata* with a tryptophan content of 3.0 g (100 g protein)$^{-1}$, while Tibbetts, Milley, and Lall [227] reports 1.5 g (100 g protein)$^{-1}$ for the Morgan-Shacklock variant of *P. palmata* and 2.6 g (100 g protein)$^{-1}$ for the wild type of *P. palmata*. Thus, in both these studies, tryptophan concentrations in *P. palmata* exceed the minimum requirement of the scoring pattern. Cysteine concentrations in *P. palmata* were reported by Tibbetts, Milley, and Lall [227] as 3.6 g (100 g protein)$^{-1}$ for the Morgan-Shacklock variant and 3.4 g (100 g protein)$^{-1}$ for the wild type. Thus for *P. palmata*, the sulphur amino acids (MET and CYS) would be the limiting amino acids regarding the scoring pattern. Therefore, our amino acid scores may have been different, had we quantified tryptophan and cysteine. Since these amino acid scores represent a theoretical value, further studies are needed to assess the true ileal digestibility of these seaweed species. However, with amino acid scores close to or over 100 % (except for *L. solidungula*), all investigated seaweeds are good candidates for a digestibility study.

Palmaria palmata had the highest concentrations of the two amino acids ASP and GLU, which are associated with umami taste when in their free form [148]. Mouritsen et al. [149] also found extracts from *P. palmata* to have comparable ASP and GLU concentrations to extracts made from brown seaweeds normally considered to be high in these components. However, we determined the total amino acid content, which may differ from the free amino acid content.

Principal component analysis of the amino acid pattern revealed no separation between fucoids or kelp species (see figure 6.3, page 66). *Hedophyllum nigripes* mainly differed along PC2 (most strongly negatively influenced by the hydroxyproline (HYP) concentration) from the other seaweeds, and *P. palmata* differed along both PC1 (most strongly influenced by both the glycine (GLY) and valine (VAL)) contents) and PC2 (most strongly influenced by the cystine (CC) content), see figure 6.4, page 67. While we did not investigate enough samples from any location to produce results that would allow statistically valid conclusions, we found it valuable to include the sample location in figure 6.3 (page 66). From the results of the PCA, it seems that the variation in amino acid profile within sampling location is at least as great as between species. However, to obtain robust results, a thorough investigation of location differences should be carried out. Bak [10] found no difference between cultivation sites in the amino acid profiles of Faroese *S. latissima*, but their sites were within a few kilometres of each other.

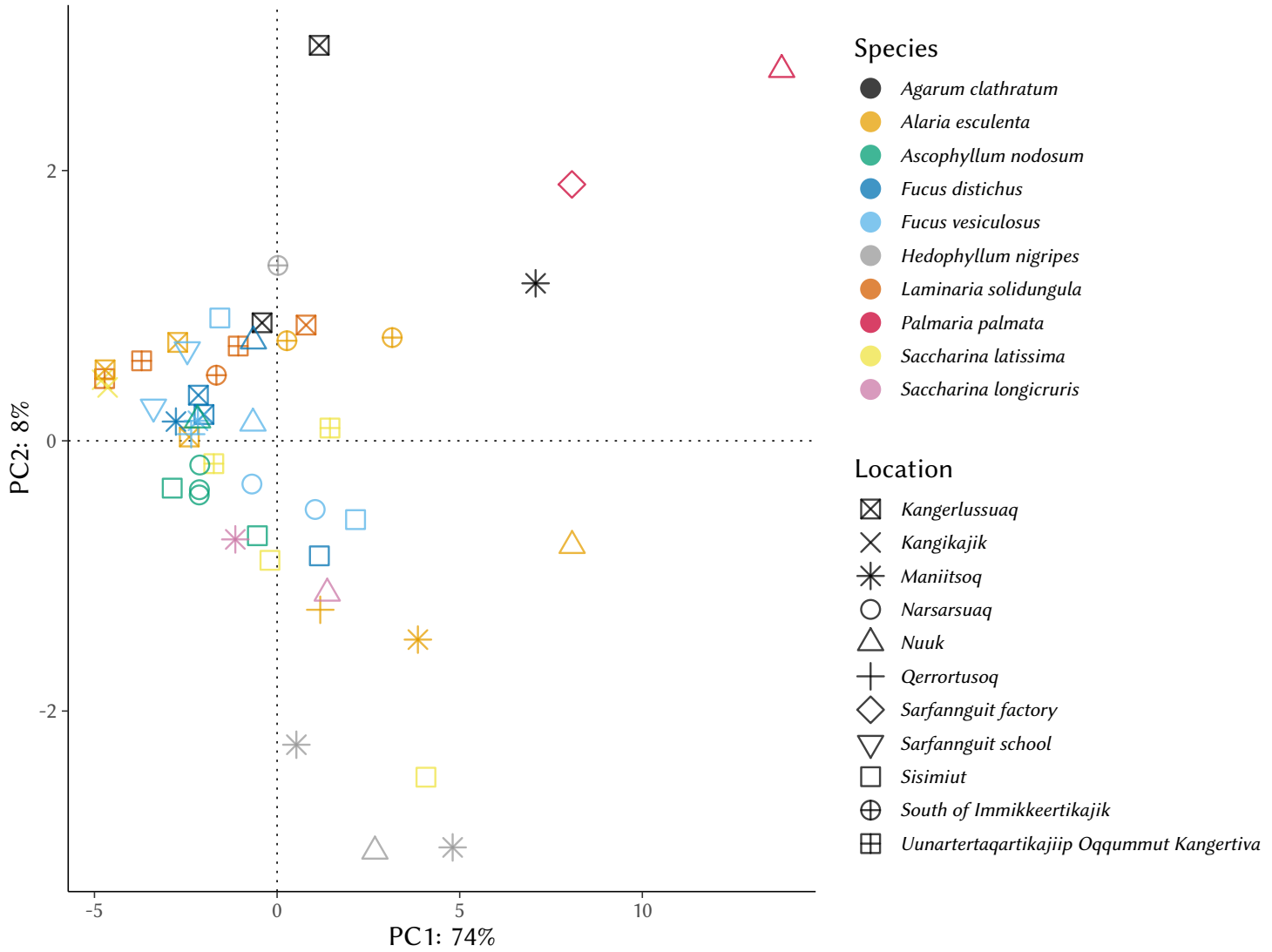

Figure 6.3: Principal component analysis of amino acid composition of seaweeds from Greenland. Each symbol represents one sample, and therefore no statistically valid conclusions on location differences can be drawn.

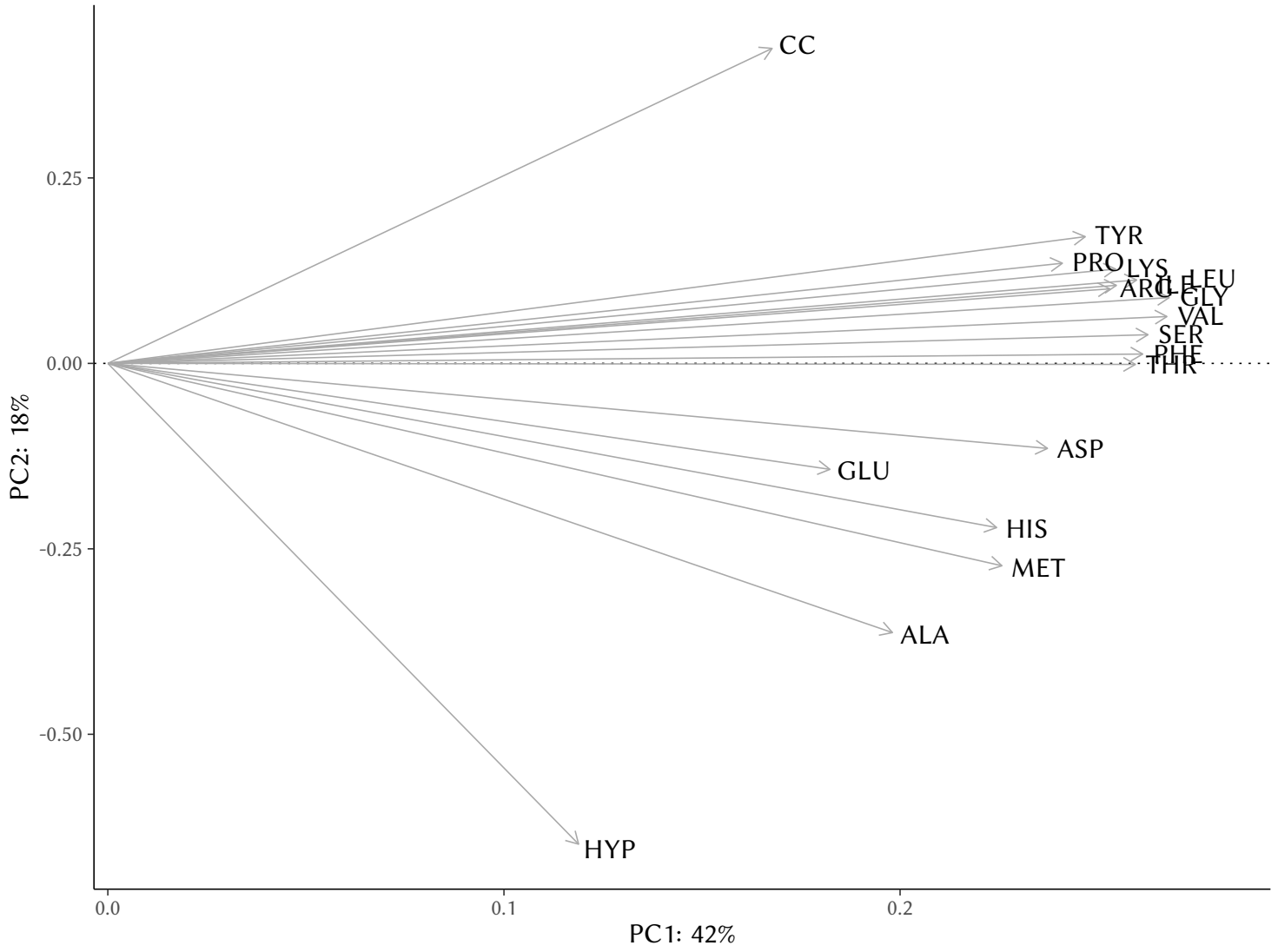

Figure 6.4: Principal component analysis loadings plot of amino acid composition of seaweeds from Greenland.

6.4.4 Antioxidant concentrations and activity

Methanolic extracts from fucoids showed the highest antioxidant concentrations (TPC) and activity (DPPH, expressed as EC_{50}), see table 6.6 (page 68). Extracts from *A. esculenta*, *H. nigripes*, *L. solidungula* and *S. latissima* had statistically significant lower DPPH activity than those from fucoids. *A. esculenta*, *H. nigripes*, *L. solidungula* and *S. latissima* had statistically significant lower TPC contents than *A. nodosum* and *F. vesiculosus*, while *F. distichus* was only statistically significantly higher in content than *L. solidungula* and *S. latissima*.

Table 6.6: DPPH radical scavenging ability (expressed as EC_{50}) and total phenolic content (TPC) for seaweeds from Greenland. Results are expressed as median concentration ± median absolute deviation.

Species	n	EC_{50} (mg extract mL^{-1})	TPC (µg GAE g^{-1} dry weight)
Agarum clathratum	3	0.21 ± 0.17	8.33 ± 6.39
Alaria esculenta	8	0.43 ± 0.24	3.80 ± 2.13
Ascophyllum nodosum	6	0.04 ± 0.01	8.37 ± 2.23
Fucus distichus	5	0.06 ± 0.03	6.18 ± 3.14
Fucus vesiculosus	9	0.03 ± 0.01	12.50 ± 8.08
Hedophyllum nigripes	4	2.54 ± 0.42	1.09 ± 0.28
Laminaria solidungula	5	2.49 ± 1.56	0.57 ± 0.14
Palmaria palmata	2	2.33 ± 1.06	2.48 ± 1.11
Saccharina latissima	6	0.77 ± 0.69	1.73 ± 0.63
Saccharina longicruris	2	0.53 ± 0.50	1.92 ± 0.15

It is difficult to compare absolute values obtained with antioxidant assays with published literature, since different extraction methods and solvents produce different results (see Wang et al. [230] on *P. palmata*). Therefore, figure 6.5 (page 69) shows a qualitative literature comparison. We have compared the median results of our study with those by Boisvert et al. [24], Farvin and Jacobsen [76], O'Sullivan et al. [168], Roleda et al. [190], Tibbetts, Milley, and Lall [227], and Wang et al. [230], who studied two to four of the same species. The general sequence for DPPH of fucoids < *A. clathratum* < kelp species & *P. palmata* was also reflected in the other studies. Similarly, the inverse was true for TPC. Jeon et al. [104] was the only study we found which investigated antioxidant activity in *A. clathratum*. They reported a DPPH radical scavenging activity inhibition of 61.5 ± 1.3 % at 40 µg mL^{-1} ethanol extract concentration, which, assuming a linear correlation, can be recalculated to an EC_{50} of 32.5 µg mL^{-1} ethanol extract. This is a more effective radical scavenging activity than what we observed with 132 ± 77 µg mL^{-1} methanol extract.

The objective of our study was to investigate the range of antioxidant activity in the investigated whole seaweed, for which methanol extracts were chosen since methanol is an effective solvent. For seaweed extracts to be used as food ingredients (see e.g., [40, 94]), another solvent

such as ethanol or water, should be chosen.

DPPH general trend: Fucoids < *Agarum clathratum* < kelp species & *Palmaria palmata*

●*F. vesiculosus* < ●*A. nodosum* < ●*F. distichus* < ●*A. clathratum* < ●*A. esculenta* < ●*S. longicruris* < ●*S. latissima* < ●*P. palmata* < ●*L. solidungula* < ●*H. nigripes* - this study

●*F. vesiculosus* < ●*A. nodosum* - O'Sullivan et al. [168]
●*F. vesiculosus* < ●*F. distichus* < ●*P. palmata* < ●*S. latissima* - Farvin and Jacobsen [76]
●*A. nodosum* < ●*S. longicruris* - Boisvert et al. [24]
●*P. palmata* < ●*S. latissima* < ●*A. esculenta* - Wang et al. [230]
●*F. vesiculosus* < ●*A. nodosum* < ●*A. esculenta* < ●*S. latissima* < ●*P. palmata* - Roleda et al. [190]

TPC general trend: Fucoids > *A. clathratum* > kelp species & *P. palmata*

●*F. vesiculosus* > ●*A. nodosum* > ●*F. distichus* > ●A. clathratum > ●*A. esculenta* > ●*P. palmata* > ●*S. latissima* > ●*S. longicruris* > ●*H. nigripes* > ●*L. solidungula* - this study

●*A. nodosum* > ●*F. vesiculosus* - O'Sullivan et al. [168]
●*F. vesiculosus* > ●*F. distichus* > ●*P. palmata* > ●*S. latissima* - Farvin and Jacobsen [76]
●*A. nodosum* > ●*S. longicruris* - Boisvert et al. [24]
●*A. nodosum* > ●*F. vesiculosus* > ●*A. esculenta* > ●*S. latissima* > ●*P. palmata* - Tibbetts, Milley, and Lall [227]
●*A. esculenta* > ●*S. latissima* > ●*P. palmata* - Wang et al. [230]
●*F. vesiculosus* > ●*A. nodosum* > ●*A. esculenta* > ●*S. latissima* > ●*P. palmata* - Roleda et al. [190]

Figure 6.5: Qualitative comparison of DPPH and TPC results of this study on seaweeds from Greenland with other studies.

Low DPPH radical scavenging properties (i.e., high EC_{50} values) correlated with low TPC concentrations, as shown in figure 6.6 (page 70). However, extracts from both fucoids and *A. clathratum* showed high DPPH radical scavenging properties (low EC_{50} values) over a wide range of TPC concentrations. Therefore, we conclude that the free radical scavenging property of these extracts is also positively influenced by other scavenger components than phenolics and what is measured with TPC. It is a known limitation that the TPC assay is non-specific for phenols and will also include non-phenolic reducing agents, such as tyrosine.

6.4.5 Greenland seaweeds are a nutritious food item

Palmaria palmata had the highest protein content (12 ± 3 %) and total content of amino acids of interest for umami flavour (GLU and ASP), as well as the highest content of eicosapentaenoic acid (EPA) 20:5(n-3). Like the fucoid species, it is also easily accessible at low tide for harvesting, and easily identified. It is therefore an interesting species to use as food.

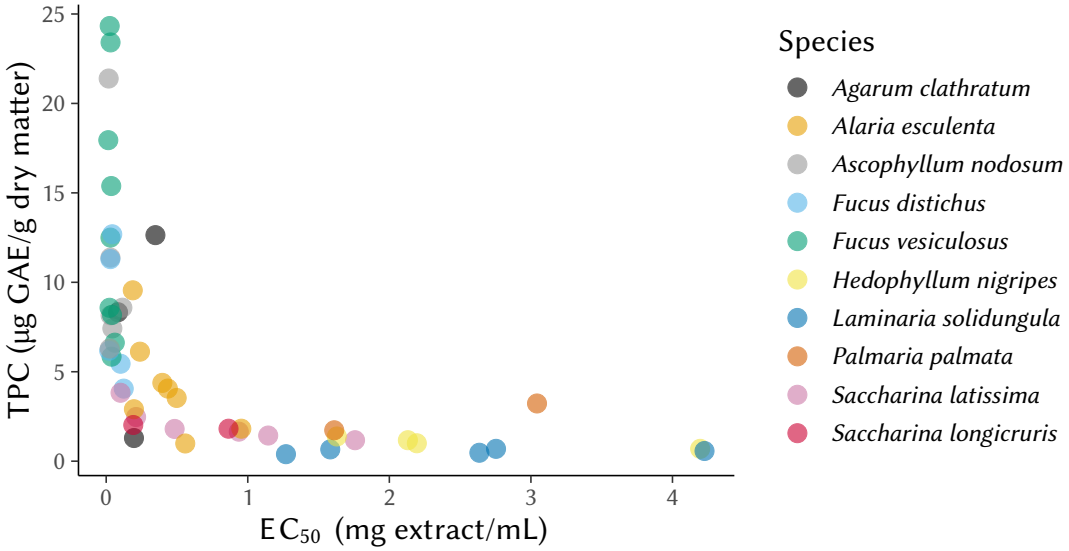

Figure 6.6: DPPH radical scavenging property (as EC_{50}) plotted against total phenolic content (TPC) for each sample of seaweeds from Greenland. Both assays were conducted from the same methanol extract.

Fucoids (*A. nodosum*, *F. distichus* and *F. vesiculosus*) showed the highest antioxidant activity (with EC_{50} ranging from 0.03 ± 0.01 mg extract mL^{-1} to 0.06 ± 0.03 mg extract mL^{-1} and TPC content ranging from 6.18 ± 3.14 µg GAE g^{-1} dw to 12.50 ± 8.08 µg GAE g^{-1} dw). Of the species studied, fucoids, and especially *F. vesiculosus*, had the highest contents of polyunsaturated fatty acids with dietary relevance for humans (linoleic acid 18:2 (n-6), α-linolenic acid 18:3 (n–3), arachidonic acid 20:4 (n-6) and docosahexaenoic acid (DHA) 22:6 (n-3)), and are therefore the most promising candidates for the use as functional food ingredients. These three species are furthermore easily accessible at low tide for harvesting.

Kelp species (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*) had the lowest dry matter content of the studied species. Compared to fucoids, they are not interesting sources for antioxidants based on the results from the assays used in this study. They had good n-6/n-3 ratios and protein contents of around 6 % dw.

Agarum clathratum, although in the order of Laminariales, was not comparable in its composition with the other four species from the same order, here described as kelps (*H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*). Indeed, it had a unique fatty acid and amino acid profile pattern. With regards to antioxidant activity, *A. clathratum* was comparable to the investigated fucoids. *Agarum clathratum* could therefore be an interesting candidate for the extraction of antioxidants.

Location may play a role in nutritional composition, but further research is necessary to

provide statistically significant results.

A barrier to commercial exploitation is however, that *A. clathratum*, *F. distichus*, *H. nigripes* and *L. solidungula* fall under the category of novel foods and would therefore require certification for the EU market.

6.5 Conclusion

In this study, 50 samples of ten different seaweed species from Greenland were investigated. The composition of these seaweed species was comparable to the composition of the same seaweed species harvested in other Nordic countries. This is with reservations in the case of *A. clathratum*, for which we found only two other studies, which were very limited in their scope. Location may have an influence on the nutritional composition and should be investigated in more detail.

Several of the investigated seaweeds are good candidates for the extraction of antioxidants (*A. clathratum*, *A. nodosum*, *F. distichus* and *F. vesiculosus*) while the other species, especially *P. palmata*, are interesting for human consumption. The novel food regulation may pose a barrier to the EU market, requiring certification for some species (*A. clathratum*, *F. distichus*, *H. nigripes* and *L. solidungula*).

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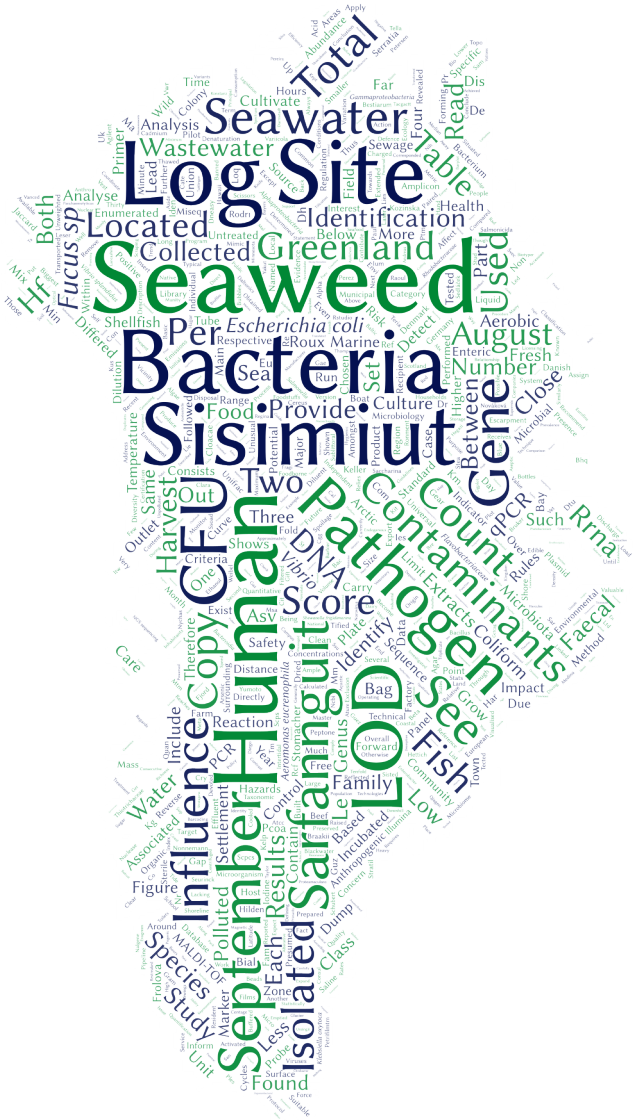
6.8 Author contributions

CRediT (Contributor Roles Taxonomy) author contributions
KJK - Conceptualisation, data curation, formal analysis, funding acquisition, investigation, project administration, visualisation, writing - original draft
LTH - Conceptualisation, funding acquisition, project administration, resources, supervision, writing - review & editing
SW - Funding acquisition, resources, supervision, writing - review & editing
OG - Funding acquisition, resources, supervision, writing - review & editing

CJ - Conceptualisation, funding acquisition, resources, supervision, writing - review & editing
SLH - Conceptualisation, funding acquisition, resources, supervision, writing - review & editing

7 The influence of anthropogenic contamination



Publication information

Microbiota of bladderwrack harvested in and outside areas impacted by human sewage in Greenland

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7.1 Abstract

Seaweed from Greenland has potential as a food source. However, as human sewage is discharged directly to the sea in the vicinity of the communities, it is necessary to understand if the microbiota of seaweed harvested close to or further away from such discharges would be impacted by human pathogens to inform future guidelines for locations suitable for harvest and cultivation. This study investigated the microbiota of *Fucus* sp. samples collected from three different sites (two presumed clean and one close to the wastewater outlet) in Sarfannguit, a smaller settlement, and two sites close to municipal wastewater outlets at Greenland's second biggest town, Sisimiut. The faecal indicator bacteria *Escherichia coli* and coliforms were detected on seaweed and in seawater obtained in Sisimiut, while only samples harvested close to the dump site in Sarfannguit tested positive. Neither *E. coli*, coliforms nor total aerobic counts showed a consistent relationship between the seaweed and the surrounding seawater. Quantification through 16S rRNA qPCR revealed bacterial abundance of 8.9 ± 0.1 to 10.1 ± 0.0 log gene copies g^{-1} seaweed. While MALDI-TOF MS analysis of bacterial isolated identified human pathogens, no evidence of the human faecal marker HF183 was detected in samples from Sarfannguit while samples from Sisimiut tested positive. Analysis of the seaweed microbiota identified the main bacterial classes as *Alphaproteobacteria*, *Bacteroidia* and *Gammaproteobacteria*, and the two major bacterial families as *Flavobacteriaceae* and *Rhodobacteraceae*. In conclusion, we detected human pathogens on the seaweed and therefore recommend the establishment of exclusion zones around wastewater outlets, where seaweed harvesting is banned.

7.2 Introduction

Seaweeds can become a valuable food source for Greenland, both for local consumption and for export. Greenland has an extended coastline of approximately 44 000 km [26], and ample areas that could be suitable for seaweed harvest and/or cultivation. Several seaweed species of interest for human consumption can be found in Greenland waters [4, 114, 240]. While harvest of some wild seaweed species such as the sublittoral *Saccharina latissima* (sugar kelp) requires a boat, and harvesting or diving gear, other edible species such as *Fucus* sp. grow in

the intertidal zone and are accessible directly from the shoreline, also close to settlements.

Wastewater treatment is non-existent in Greenland. Untreated wastewater is thus discharged untreated into recipients such as the sea, bays or fjords from each of the communities, which range in size from 10 to 18 000 people [26, 35]. The environmental impact is believed to be low due to the coastal location of communities and low population density. However, direct discharge of untreated wastewater is likely to contribute microbial and chemical contaminants into the Arctic recipient [87, 103, 159] and can lead to public health concerns [42]. It is not known how far the microbial pollution from untreated wastewater emissions reaches and how it affects the food safety of seaweeds harvested in the proximity of human settlements.

Seaweeds have an associated epiphytic bacterial community [59, 99, 211], which amongst others, often consists of *Gammaproteobacteria* and *Alphaproteobacteria*. This resident microbial biofilm is important for the health and defence of its host [59, 99, 211] and may also affect the ability of human pathogenic bacteria to become associated with the algae. An assessment of risks associated with seaweed production led to the identification of four major hazards, namely arsenic, cadmium, iodine and *Salmonella* [11]. Only iodine was found to be an issue in our recent study, which analysed the content of microelements in 10 different species of Greenlandic seaweed [114]. *Salmonella* sp. are human enteric pathogenic bacteria and commonly linked to faecal environmental contamination. An outbreak of salmonellosis has been linked to a seaweed farm on Hawaii [160, 183]. Other foodborne pathogens have been identified as potential hazards in fresh seaweed, including norovirus [173, 212], *Vibrio parahaemolyticus* [12, 137], *Bacillus cereus* [37] and *Escherichia coli* [12]. Overall there is a knowledge gap of potential microbial hazards associated with seaweed production.

The Government of Greenland has set out some basic rules about licensing of companies harvesting or cultivating seaweed. These rules include the requirement to evaluate the risk of possible presence of health hazards of endogenous and anthropogenic origin, however there is currently no specific regulation governing seaweed utilization. Seaweed that is to be exported to the European Union would generally have to comply with the EU regulations, i.e., EC No. 2073/2005 concerning microbiological criteria for foodstuffs [66]. Although a food category devoted to seaweed is not indicated in this regulation.

In Europe, there exist very few rules and guidelines defining suitable hygienic seaweed harvest or cultivation areas [14]. Barbier et al. [14] even suggest that it would be unnecessary to monitor *E. coli* as part of a future European ecological seaweed regulations, since seaweed does not accumulate bacteria and viruses in the way that filtering species like bivalves do. In Denmark, the Danish Food Authorities studied *E. coli* and *Salmonella* on a range of seaweed species in 2017, and concluded that there were no specific areas deemed unsafe for seaweed harvest, except for in the vicinity of pollution sources such as sewage disposal points and harbours [44]. A Danish guide to organic seaweed certification recommends the description of distance to potential pollution sources in the self-control program [39]. In Scotland (United Kingdom), the Seaweed Cultivation Policy Statement (SCPS) points out the contamination risk for seaweed from sewage and other effluents. The SCPCS recommends permitted seaweed cultivation sites to be situated within designated shellfish waters which are monitored with

regards to the impact of water quality on the safety of the produced food [204].

Knowledge about the risk and prevalence of enteric pathogens associated with seaweeds harvested close to communities in Greenland is lacking. To address this, the present study investigated the influence of wastewater emissions on the microbial community of wild *Fucus* sp. in Greenland. We compared the content of faecal indicator bacteria and the microbiota on collected seaweed and in seawater samples from sewage impacted sites in two settlements of different sizes: Sisimiut and Sarfannguit, situated within 40 km of each other, in August and September 2017, and in August 2018. Samples from a non-sewage impacted site in Sarfannguit were also retrieved for comparison.

7.3 Materials and methods

7.3.1 Samples and sampling locations

Fucus sp. were collected in 2017 and 2018 at low tide in Greenland. The two study sites consisted of Sisimiut, which is the second biggest town in Greenland with over 5 000 inhabitants, and Sarfannguit, which is a smaller settlement of around 100 inhabitants [26]. Both sites lie in Qeqqata municipality on the West Coast of Greenland. Seaweed and water samples were collected using aseptic techniques at two (Sisimiut) and three sampling (Sarfannguit) locations (see table 7.1, page 77). The seawater (1 L) was collected into presterilized Nalgene bottles (VWR, Denmark), and the seaweed (approximately 500 g, 3 to 4 individuals) was collected into sterile stomacher bags (Fisher Scientific, Denmark) after cutting the algae with sterile (70 % v/v ethanol) scissors or a knife.

In Sisimiut, a large part of the households discharge their blackwater at the dump, while Sarfannguit relies on bag toilets which are collected and emptied on land at the escarpment dump site. The escarpment site had been chosen so that the waste must percolate more than 100 metres downhill through soil before reaching the sea, which the inhabitants expect to reduce the impact on the recipient (P. E. Jensen, personal communication, February 5th, 2021). The sampling locations in Sisimiut in Kangerluarsunnguaq bay and close to the dump outlet were chosen as representatives for a heavy blackwater pollution. The dump and fish factory sampling locations in Sarfannguit were chosen as representatives of the human sewage and industrial pollution, respectively, in a smaller settlement, with the school location chosen as an unpolluted and presumed pristine location with no obvious influence from pollution point sources.

Sampling was performed in August and September (only Sisimiut) in 2017 and in September 2018. Samples were kept in a cooler (maximum 5 ℃) and transported to the DTU Sisimiut Campus laboratory located in Sisimiut and processed for microbiological analyses (see section 7.3.2, page 77) within 24 hours of sampling (Sisimiut samples) or 24 to 36 hours of sampling (Sarfannguit samples). Fresh seaweed samples were transported cooled back to the Technical University of Denmark's main campus in Kgs. Lyngby for analyses through MALDI-TOF in September 2018.

Table 7.1: Seaweed and seawater sampling locations in Sarfannguit and Sisimiut, Greenland. Coordinates in decimal degrees.

Location	Site	Site description	Number of samples	Latitude	Longitude
Sarfannguit	A	below escarpment of waste dump	2 seaweed, 2 seawater	66.897536	-52.874138
	B	close to fish factory	2 seaweed, 2 seawater	66.898226	-52.858431
	C	close to school	2 seaweed, 2 seawater	66.896311	-52.857659
Sisimiut	D	shore close to dump	3 seaweed, 3 seawater	66.928316	-53.673514
	E	shore close to wastewater outlet	3 seaweed, 3 seawater	66.943028	-53.651677

7.3.2 Preparation of seaweed samples for culture and culture-independent microbiological analyses

To remove microorganisms, 20 g seaweed was mixed with 20 mL phosphate buffered saline (for 2017 samples) or 20 mL peptone saline (for 2018 samples) in a stomacher bag and massaged by hand for one minute. This manual method was confirmed as robust alterative to using a stomacher in a pilot study (data not shown).

7.3.3 Enumeration of total aerobic count, coliforms, and *Escherichia coli*

The total bacterial load and faecal bacterial load in the seaweed and seawater samples were enumerated on Petri films. Briefly, for each site, one mL of the dilution liquid (and subsequent tenfold dilutions) from the stomacher bag, or one mL of seawater was inoculated on 3M™ Petrifilm™ Aerobic Count Plates (AC) (3M, St. Paul, Minnesota, USA) and 3M™ Petrifilm™ *E. coli*/Coliform Count Plate (EC) according to manufacturer's instructions. In 2017, the AC samples were incubated at 3 ℃ to mimic environmental temperatures. However, since the incubation at 3 ℃ extended the incubation time to 7 days to produce readable results, in 2018, the incubation temperature was raised to 35 ℃. EC plates were incubated at 35 ℃ or 37 ℃. All or typical blue and/or red colonies with gas bubbles were counted on the AC and EC plates after incubation for 48 (168 for the low incubation temperature) and 24 hours, respectively, and reported as colony forming units (CFU) per g seaweed or ml seawater.

7.3.4 Identification of seaweed bacteria using matrix-assisted laser desorption/ionization - time-of-flight (MALDI-TOF)

In 2018, bacterial colonies were picked from AC plates transported to Sisimiut and subcultured on marine agar (MA, PanReac AppliChem ITW Reagents 414680.1210, Darmstadt, Germany). Single colonies (up to thirty per seaweed sample location) were isolated, restreaked on MA to cultivate and check purity (15 ℃, 3 days) and preserved by scraping colony mass into cryopreservation beads (Microorganism Preservation System – Protect, Technical Service Consultants Ltd, Lancashire, UK) for long-term storage at -80 ℃. Prior to identification by MALDI-TOF,

isolates were thawed and recultured on marine agar at 15 ℃ for 3 days. Colonies from fresh plates were extracted with the ethanol/formic acid/acetonitrile protocol used by Nonnemann et al. [163], as described by Bizzini et al. [20]. Mass spectra were produced with Autoflex Speed instrument (Bruker Daltonics, Billerica, Massachusetts, USA) and identified using the Technical University of Denmark, National Veterinary Institutes Main Spectrum Database (DTU-Vet MSP Database) as described in Nonnemann et al. [163].

7.3.5 Culture-independent microbiology

Deoxyribonucleic acid (DNA) extraction

DNA was extracted from a liquid subsample from the stomacher bags (seaweed and diluent) and used for culture-independent analysis of the microbiota and quantitative PCR (qPCR) based enumeration of the total bacterial count (16S rRNA gene copy numbers, [159]) and gene copies of the HF183 marker, which detects the 16S rRNA of human *Bacteroidales* as a specific indicator of human faecal contamination [206]. Two mL of the seaweed-diluent mix was used for DNA extraction, and centrifuged at 9 614 relative centrifugal force (rcf) (Hettich Mikro 185, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) for 10 minutes. The supernatant was carefully removed, and the pellet was resuspended in 200 µL of the remaining liquid in the tube. These 200 µL were then transferred to the DNEasy PowerBead tube of the Qiagen DNEasy PowerSoil Kit (Quiagen, Hilden, Germany). DNA extraction was carried out following the manufacturer's protocol. A field control sample was included by extracting DNA from 200 µL of the diluent containing no seaweed. All DNA extracts were stored at -20 ℃ until analyses. The extracted environmental DNA from seaweed was analysed quantitively by qPCR and qualitatively by 16S rRNA amplicon sequencing of the microbiota on the Illumina Miseq platform (see below, seawater samples were not analysed). Table 7.2 (page 78) lists the primer sets and purpose of the different methods.

Table 7.2: List of culture-independent methods, gene targets, and primers ('5 – 3') used to analyse DNA extracted from Greenlandic *Fucus* sp. seaweed.

Method		Purpose	Primer and probe	Reference
qPCR	16S rRNA	Total bacterial count	Forward: CGGTGAATACGTTCYCGG , Reverse: GGWTACCTTGTTACGACTT	Suzuki, Taylor, and DeLong [222]
	HF183	Human faecal marker	Forward: ATCATGAGTTCACATGTCCG, Reverse: CTTCCTCTCAGAACCCCTATCC, Probe: FAM-CTAATGGAACGCATCCC-BHQ1	Seurinck et al. [206]
NGS	16S rRNA	Bacterial community (V1-V3)	Forward: AGAGTTTGATCATGGCTCAG, Reverse: GTATTACCGCGGCTGCTG	Leser et al. [128] and Weisburg et al. [238]

Enumeration of the culture-independent total bacterial community and presence of human faecal bacteria by qPCR.

Quantification of 16S rRNA and HF183 gene copy numbers were performed in optical tubes and caps (Agilent Technologies, Santa Clara, California, USA) on a Stratagene Mx3005P™ qPCR System (Agilent Technologies). Each sample run included positive plasmid standards (see below), non-template controls and water only wells. All samples were determined in technical duplicates.

For enumeration of the 16S rRNA gene copy numbers, each qPCR reaction consisted of: 9.5 µL nuclease free water, 12.5 µL EvaGreen® master mix (Qiagen, Hilden, Germany), 1 µL of each of 0.4 µM, forward and reverse primers and 1 µL of sample DNA for a total reaction volume of 25 µL. The conditions for the qPCR reaction were: initial denaturation for 5 min at 95 ℃, followed by 40 cycles of 30 s at 95 ℃, 30 s at 50 ℃, and 30 s at 68 ℃, then 5 min at 68 ℃. During the melting curve analysis, the temperature was increased from 45 to 95℃. Gene copies per gram seaweed were calculated based on a standard curve that was constructed using 10-fold dilutions of a plasmid DNA extracted from an E. coli DH5α culture containing a positive control plasmid (pCR2.1, TOPO TA PCR 2.1) with the inserted gene of interest (gift from Dr. C. Yost, University of Regina, Canada). The standard curve was determined from triplicate measurements of 1 to 1×10^9 gene copies per reaction. The qPCR efficiency was 113 %, with R^2 value of 0.993. The limit of quantification (LOQ) and limit of detection (LOD) were 10^4 and 10^3 gene copies per reaction, respectively. In the seaweed samples this corresponded to a LOD of 10^5 gene copies g^{-1}.

For the quantification of the HF183 marker of human faecal contamination, the qPCR reaction was composed of: 6.875 µL nuclease free water, 12.5 µL TaqMan® master mix (SsoAdvanced Universal Probes Supermix, Bio-Rad, Hercules, California, USA), 1.5 µL each of 0.6 µM forward and reverse primers, 0.625 µL Taqman probe specific for human *Bacteroidales* 16S rRNA [125, 206] and 2 µL of sample DNA. The total volume of the reaction was 25 µL. The conditions for the PCR were: initial denaturation for 3 min at 95 ℃, followed by 40 cycles of 30 s at 95 ℃ and 30 s at 58 ℃, then 5 min at 68 ℃. The positive control consisted of plasmid DNA standard, which was extracted from an E. coli DH5α strain with pCR2.1 with the HF183 insert (see Stea et al. [215]). The standard curve was built by qPCR analysis in triplicates of 10-fold dilutions of the plasmid (4 to 4×10^8 gene copies per µL). All samples were determined in technical duplicate. The obtained standard curve had an efficiency of 105 %, with an R^2 value of 0.991. Both the LOQ and LOD were 3.9 gene copies per reaction, respectively. This resulted in an LOD in the seaweed samples 10^2 gene copies g^{-1}.

Illumina meta-barcoding sequencing

The seaweed microbiota was analysed using 16S rRNA amplicon sequencing. Libraries were prepared from aliquots of 20 µL DNA using the pipeline of Eurofins Genomics (Konstanz, Germany), followed by sequencing of a target region of the V1-V3 part of the 16S rRNA gene

(see table 7.2, page 78) on an Illumina MiSeq platform (Illumina, San Diego, California, United States). Along with the samples, the negative field control sample (see above, no seaweed only diluent) and a mock community (ATCC® MSA-1000™) were sequenced. Eurofins performed quality control check and trimmed the primer sequence region from the reads. Paired-end reads were deposited at the NIH NCBI Sequence Read Archive with the accession number PRJNA699133.

7.3.6 Data analysis

Data was analysed and visualised with RStudio version 1.1.463 [193] with R version 3.4.4 (2018-03-15) [181], transformed using dplyr [243], statistically analysed with the stats package [181], and visualised with ggplot2 [241], unless otherwise noted.

MALDI-TOF-MS

Log scores between 0 and 3 were calculated by the Biotyper algorithm (Bruker Daltonics, Bremen, Germany). Log scores <1.7 do not lead to identification. Log score 1.7 ≥ x < 2.0 provides genus identification, and log score ≥2 provides species identification. Log scores ≥2.0 provide specific species identification.

NGS results

Quantitative Insights Into Microbial Ecology 2 (QIIME2) [27], the DADA2 pipeline [32] and the standard operating procedure Amplicon SOP v2 [50] were used to assign Amplicon Sequence Variants (ASV) from the assembled paired-end reads. To minimise sequencing carry-over contamination between MiSeq runs, ASVs with an abundance of less than 0.1 % of the total observations were filtered out. The minimum sampling depth of the analysis was set to 38 000 reads, based on the number of reads in the sample with fewest reads, aside from the negative field control, which had less than 3000 reads. To assign a taxonomical classification to the ASVs, a Native Bayers trained classifier was obtained from the SILVA 138 SSU Ref NR 99 database [25, 180].

Alpha diversity was investigated with the "taxa barplot" function. Beta diversity was investigated with the Jaccard similarity index "–p-metric: jaccard", subsequently translated to Jaccard distance and unweighted UniFrac with "–p-metric: unweighted_unifrac" (based on the qualitative abundance of ASVs in samples).

7.4 Results and discussion

7.4.1 Total aerobic count, coliforms, and *Escherichia coli*

The results of bacterial counts are shown in table 7.3 (page 81). Seaweed samples from Sisimiut tended to have higher CFU 35 °C aerobic counts (over 7.4 log CFU g^{-1} or mL^{-1}) than those from

Sarfannguit (below 6.5 log CFU g^{-1} or mL^{-1}, except for site A in September 2018). However, there was considerable variation at the same sampling site when sampling in two consecutive months in 2017, or between years when sampling in the same month.

Table 7.3: Colony forming unit (CFU) counts for aerobic counts, coliforms and *E. coli* from *Fucus* sp. and seawater samples from Greenland. Counts are expressed as log CFU g^{-1} or mL^{-1}.

Site			Aerobic count 3 ℃	Aerobic count 35 ℃*	Coliforms	*E. coli*	Month	Year
Seaweed samples	Sarfannguit	A	9.3	6.4	0	0	August	2017
			NA**	7.5	<LOD***	<LOD	September	2018
		B	9.5	3.3	0	<LOD	August	2017
			NA	4.3	<LOD	<LOD	September	2018
		C	10.3	5.3	<LOD	<LOD	August	2017
			NA	5.7	<LOD	<LOD	September	2018
	Sisimiut	D	NA	7.4	7.3	6	August	2017
			NA	12.5	8.4	5.3	September	2017
			NA	12.4	7.5	4.6	September	2018
		E	NA	10.3	8	3.9	August	2017
			NA	8.7	4.8	4.2	September	2017
			NA	10.1	4.9	3.5	September	2018
Seawater samples	Sarfannguit	A	7.5	NA	5.7	5.7	August	2017
			NA	4.4	<LOD***	<LOD***	September	2018
		B	6.1	NA	<LOD***	<LOD***	August	2017
			NA	NA	<LOD***	<LOD***	September	2018
		C	8.3	NA	<LOD***	<LOD***	August	2017
			NA	3.1	<LOD***	<LOD***	September	2018
	Sisimiut	D	5.7	3.6	5	3.9	August	2017
			NA	9.6	4.8	2.1	September	2017
			NA	6.2	2.3	2.3	September	2018
		E	5.4	8.0	8.6	7.1	August	2017
			NA	9.7	6.4	5.8	September	2017
			NA	12.2	6.5	6.1	September	2018

* Total aerobic count CFU were incubated at 35 ℃ in 2018.
**NA: Not available – this analysis was not carried out.
*** LOD was 2 CFU g^{-1} or mL^{-1} in seaweed and 1 CFU g^{-1} or mL^{-1} in seawater.

Total aerobic counts from the same sample carried out at 3 ℃ and 35 ℃ showed a trend towards much higher amounts of colony forming units (CFU) at the lower incubation temper-

ature, for example 9.3 log CFU g^{-1} seaweed compared to 3.3 log CFU g^{-1} seaweed in the case of Sarfannguit fish factory August 2017, see table 7.3 (page 81). This is likely because many environmental bacteria were not able to grow or even died at the higher incubation temperatures.

For most locations in Sarfannguit, both coliforms and *E. coli* were below detection limit (<1 respectively <2 CFU mL^{-1} seawater or g^{-1} seaweed), see table 7.3 (page 81). However, when we detected coliforms or *E. coli* in the seawater samples, we always also detected them on the seaweed. In Sisimiut, the concentrations of coliforms and *E. coli* detected on seaweed samples were higher by site D (median ± median absolute deviation: 7.5 ± 0.4 log CFU g^{-1} seaweed and 5.3 ± 1.0 log CFU g^{-1} seaweed) than by site E (4.9 ± 0.2 log CFU g^{-1} seaweed and 3.9 ± 0.5 log CFU mL^{-1} seaweed). The inverse situation was the case for the seawater samples, with lower coliform and *E. coli* counts at site D (4.8 ± 0.4 CFU mL^{-1} seawater and 2.3 ± 0.3 CFU mL^{-1} seawater) compared to site E (6.5 ± 0.2 CFU mL^{-1} seawater and 6.1 ± 0.5 CFU mL^{-1} seawater). We theorise that this is due to the much higher water exchange rates at site D, which lies exposed towards the open sea. Site E is in the more sheltered Kangerluarsunnguaq Bay. Barberi et al. [12] also found that bacterial plate counts from seaweed often differed from those of the surrounding water.

7.4.2 Identity of commensal bacteria *Fucus* sp. samples as determined by MALDI-TOF

A total of 156 bacteria were isolated from *Fucus* sp. samples collected in September 2018, with 64 derived from samples from Sisimiut and 92 from Sarfannguit. A further 17 bacteria were isolated from the seawater sampled in Sisimiut and 20 from Sarfannguit. While 40 % to 88 % of the seaweed isolates could be identified from both sites D and E in Sisimiut with more intensive pollution, the method was far less successful for identification of organisms from the less polluted sites (A, B and C) in Sarfannguit (see table 7.4, page 83 and table 7.5, page 85), where 6 % to 24 % were identified. Similarly, 100 % of the seawater isolates from Sisimiut could be identified, in contrast to only 11 % of those from Sarfannguit.

Several human pathogens, such as *Klebsiella oxytoca* or *Aeromonas eucrenophila* could be identified from the seaweed, see table 7.4, page 83. Seawater samples from Sisimiut also showed evidence of human contamination, as exemplified by the isolation of *E. coli*, see table 7.5, page 85. The influence of the fish processing in the factory in Sarfannguit is reflected in the identification of fish pathogens *Aeromonas salmonicida* and *Vibrio splendidus*.

Though MALDI-TOF MS is a promising method to study the bacteria associated with macroalgae [61], our study shows a clear limitation of the technique as the available MALDI-TOF database was not geared to identify bacteria from the arctic marine environment. Other studies have therefore included other identification techniques such as 16S rDNA sequencing [163, 196] as well as nuclear magnetic resonance spectroscopy [196]. The library available at our institute was primarily built for veterinary and medical purposes including foodborne pathogens, and less for environmental bacteria.

Table 7.4: Bacterial isolates from seaweed from Greenland identified by MALDI-TOF MS.

Site		Organism	n*	Log score**	Classification	Reference
Sarfannguit	A	*Vibrio splendidus*	2	>2	Fish and shellfish pathogen	Le Roux et al. [126]
	B	*Acinetobacter ursingii*	1	>2	Human pathogen	Yakut et al. [245]
		Aeromonas salmonicida	1	1.7 ≥ x < 2.0	Fish pathogen	Emmerich and Weibel [62]
		Enterobacter cloacae	1	1.7 ≥ x < 2.0	Health-care associated human pathogen	Keller et al. [108]
		Pseudomonas fragi / Pseudomonas taetrolens	1	1.7 ≥ x < 2.0	Dairy and meat spoilage/mustiness in eggs	Levine and Anderson [129] and Pereira and Morgan [176]
		Serratia proteamaculans/ Serratia liquefaciens	1	>2	Minced beef spoilage	Nychas and Drosinos [167]
		Stenotrophomonas maltophilia	1	1.7 ≥ x < 2.0	Health-care associated human pathogen	Oliveira-Garcia et al. [170]
		Vibrio splendidus	1	1.7 ≥ x < 2.0	Fish and shellfish pathogen	Le Roux et al. [126]
		Vibrio splendidus / Vibrio tasmaniensis	1	1.7 ≥ x < 2.0	Fish and shellfish pathogen	Le Roux et al. [126] and Thompson and Swings [226]
	C	*Vibrio splendidus*	1	>2	Fish and shellfish pathogen	Le Roux et al. [126]
		Vibrio splendidus	1	1.7 ≥ x < 2.0	Fish and shellfish pathogen	Le Roux et al. [126]
Sisimiut	D	*Aeromonas bestiarum / Aeromonas eucrenophila*	1	>2	Fish pathogen	Kozinska and Guz [110] and Schubert and Hegazi [202]
		Klebsiella oxytoca / Raoultella ornithinolytica	2	1.7 ≥ x < 2.0	Health-care associated human pathogen	Hajjar et al. [88] and Leitner et al. [127]
		Photobacterium iliopiscarium	1	1.7 ≥ x < 2.0	Seafood spoilage	Fuertes-Perez et al. [80]
		Serratia fonticola	1	>2	Unusual human pathogen	Aljorayid et al. [1]
		Shewanella frigidimarina	4	>2	Arctic marine bacteria	Frolova et al. [79]
		Shewanella frigidimarina	1	1.7 ≥ x < 2.0	Arctic marine bacteria	Frolova et al. [79]

* Number of isolates. Total number of isolates per location: A 30, 3 of which did not grow; B 29; C 30; D 26, 1 of which did not grow; E 29.
** Log score <1.7 do not lead to identification. Log score 1.7 ≥ x < 2.0 provides genus identification, and log score ≥2 provides species identification. Log scores ≥2.0 provide specific species identification.

Continued on next page.

Bacterial isolates from seaweed from Greenland identified by MALDI-TOF MS. (Continued from previous page)

Site		Organism	n*	Log score**	Classification	Reference
Sisimiut	E	*Aeromonas eucrenophila / Aeromonas bestiarum / Aeromonas salmonicida*	1	>2	Fish pathogen	Emmerich and Weibel [62], Kozinska and Guz [110], and Schubert and Hegazi [202]
		Citrobacter freundii / Citrobacter braakii	1	>2	Unusual human pathogen	Whalen, Mully, and English [239] and Yumoto et al. [249]
		Enterobacter asburiae / Enterobacter cloacae	1	>2	Health-care associated human pathogen	Keller et al. [108] and Kus [120]
		Enterobacter cloacae	1	>2	Health-care associated human pathogen	Keller et al. [108]
		Flavobacterium glaciei	1	>2	Glacier bacterium	Zhang et al. [250]
		Klebsiella oxytoca	2	>2	Health-care associated human pathogen	Leitner et al. [127]
		Klebsiella variicola	1	>2	Human pathogen	Rodríguez-Medina et al. [187]
		Serratia proteamaculans / Serratia liquefaciens	1	>2	Minced beef spoilage	Nychas and Drosinos [167]
		Shewanella frigidimarina	2	>2	Arctic marine bacterium	Frolova et al. [79]
		Shewanella frigidimarina	1	1.7 ≥ x < 2.0	Arctic marine bacterium	Frolova et al. [79]
		Yersinia intermedia	1	>2	Fish and human enteric bacterium	Brenner et al. [28]

* Number of isolates. Total number of isolates per location: A 30, 3 of which did not grow; B 29; C 30; D 26, 1 of which did not grow; E 29.
** Log score <1.7 do not lead to identification. Log score 1.7 ≥ x < 2.0 provides genus identification, and log score ≥2 provides species identification. Log scores ≥2.0 provide specific species identification.

Table 7.5: Bacterial isolates from seawater from Greenland identified by MALDI-TOF MS.

Site		Organism	n*	Log score**	Classification	Reference
Sarfannguit	A	*Micrococcus luteus*	1	>2	Skin bacterium	Young et al. [248]
	B	No samples taken				
	C	None of the ten isolates grew				
Sisimiut	D	*Aeromonas encheleia*	1	>2	Environmental and fish bacterium	Nováková, Švec, and Sedláček [166]
	D	*Citrobakter braakii*	1	>2	Unusual human pathogen	Yumoto et al. [249]
	D	*Enterococcus faecium*	2	>2	Human pathogen	Higuita and Huycke [95]
	D	*Escherichia coli*	3	>2	Human pathogen	Petersen and Hubbart [177]
	D	*Klebsiella variicola*	2	>2	Human pathogen	Rodríguez-Medina et al. [187]
	E	*Aeromonas hydrophila*	1	>2	Environmental bacterium	Nováková, Švec, and Sedláček [166]
	E	*Cronobacter sp*	1	$1.7 \geq x < 2.0$	Human pathogen	Iversen et al. [101]
	E	*Escherichia coli*	1	>2	Human pathogen	Petersen and Hubbart [177]
	E	*Escherichia hermanii*	1	>2	Human pathogen	Ioannou [100]

* Number of isolates. Total number of isolates per location: A 9; B 0; C 10; D 9; E 8, four of which did not grow.
** Log score <1.7 do not lead to identification. Log score $1.7 \geq x < 2.0$ provides genus identification, and log score ≥2 provides species identification. Log scores ≥2.0 provide specific species identification.

This fact was reflected in our results where a high percentage of identified organisms were achieved in samples that were more impacted by anthropogenic activities, i.e., Sisimiut with 40 % to 88 % of isolates being identified versus 4 % to 24 % in samples from Sarfannguit. Therefore, we conclude that the results from our study can be a valuable addition to the other culture-dependent analysis and the NGS results, as the MALDI-TOF results support the finding of the microbiota being influenced by the surrounding water quality. Future work should expand the database to include environmental microorganisms.

7.4.3 Culture-independent microbiology in seaweed samples

The molecular total bacterial count varied from 8.9 ± 0.1 to 10.1 ± 0.0 log gene copies g^{-1} seaweed (see table 7.6) and were markedly higher than the 35 ℃ culture-based counts (see table 7.3, page 81) from Sarfannguit (sites A, B and C). This indicates that most bacteria found on seaweed in Sarfannguit were not able to grow at an incubation temperature of 35 ℃. Therefore, if Petrifilm is considered as a monitoring tool for CFU on seaweed, the incubation temperature should be adapted.

Table 7.6: Total bacterial count (16 S rRNA) and human faecal contamination indicator (HF183) results expressed as log gene copies per gram fresh seaweed (*Fucus* sp.) from Greenland.

Site		16S rRNA	HF183	Month	Year
Sarfannguit	A	9.0 ± 0.0	<LOD*	August	2017
		9.3 ± 0.1	<LOD	September	2018
	B	9.0 ± 0.1	<LOD	August	2017
		9.3 ± 0.1	<LOD	September	2018
	C	8.9 ± 0.1	<LOD	August	2017
		9.2 ± 0.0	<LOD	September	2018
Sisimiut	D	9.3 ± 0.0	3.5 ± 0.2	August	2017
		8.5 ± 0.0	3.1 ± 0.1	September	2017
		10.1 ± 0.0	4.0 ± 0.0	September	2018
	E	9.1 ± 0.0	4.0 ± 0.3	August	2017
		9.0 ± 0.1	3.5 ± 0.1	September	2017
		9.1 ± 0.1	3.4 ± 0.2	September	2018
	control	5.8 ± 0.1	<LOD	September	2018

*LOD was 2 log genes g^{-1} seaweed

We could not detect the marker of human faecal contamination, HF183, in any of the samples from Sarfannguit (below detection limit of 2 log genes g^{-1} seaweed). In contrast, HF183

concentrations in seaweed samples from Sisimiut ranged from 3.4 ± 0.2 to 4.0 ± 0.0 log gene copies g^{-1} seaweed. Since HF183 originates from anaerobic bacteria from the genus *Bacteroides*, which are optimised for human core temperature growth, our results are therefore indicative of recent contamination with human faecal waste in seaweed samples from Sisimiut.

NGS sequencing of the microbiota on *Fucus* sp.

There was little contamination of the DNA extraction protocol performed in the field environment, as evidenced in the exceptionally low number of reads of the process control (DNA extracted from peptone saline used for bacteria removal).

There was no statistically significant difference in species richness between locations with an overall species richness (number of ASVs) of 345 ± 58.

Alpha biodiversity The three major classes in all samples were *Alphaproteobacteria*, *Bacteroidia* and *Gammaproteobacteria*, see figure 7.1.

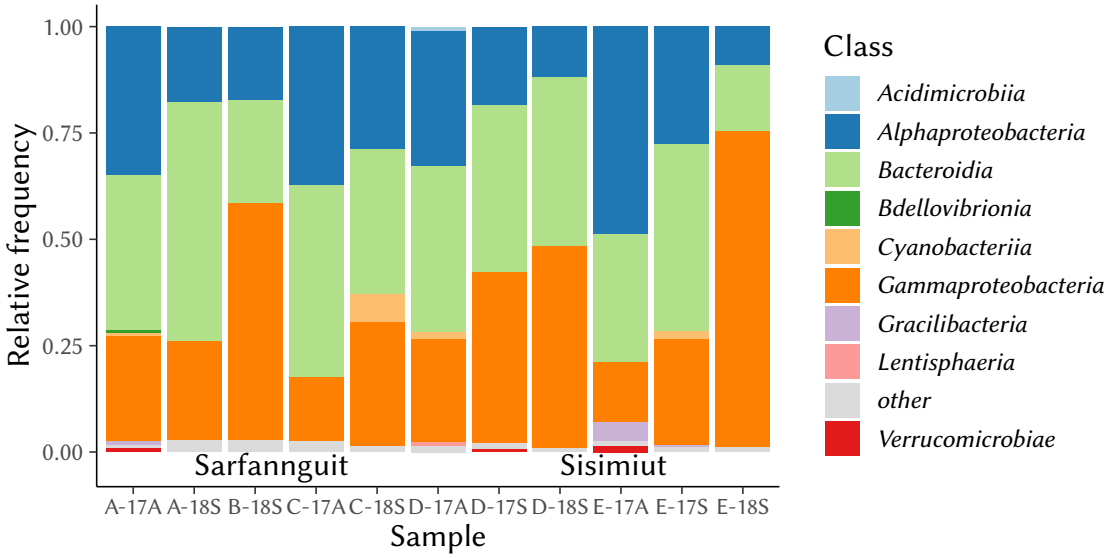


Figure 7.1: Relative abundance of surface bacterial classes across all samples of *Fucus* sp. from Greenland. Samples were from Sarfannguit (A, B and C) and Sisimiut (D and E), collected in 2017 (17) and 2018 (18), in August (A) and September (S). The category "other" contains all classes with less than 300 reads for the individual sample.

Lachnit et al. [121], Stratil et al. [219], Stratil et al. [218] and Mensch et al. [142] all reported both *Alphaproteobacteria* and *Gammaproteobacteria* as the most frequent operational taxonomic unit (OTUs) on Baltic *F. vesiculosus* collected in Kiel, Germany. Another study

from the same city found *Alphaproteobacteria* to be the dominant class [174]. Interestingly, *Alphaproteobacteria* and *Gammaproteobacteria* are also amongst the most common identified classes in a study on dried fish prepared according to Greenlandic Inuit traditions [92].

The major families identified in all samples of this study were *Flavobacteriaceae* and *Rhodobacteraceae* (see figure 7.2, page 88). *Thiotrichaceae* were only found in four of the six samples from Sisimiut. Samples from the same locations exhibited significant variation between years or even months. For example, *Thiotrichaceae* were found at the Sisismiut site E in August 2017 but not in September 2017 or September 2018. Lachnit et al. [121] similarly found that bacterial communities on *Fucus* vesiculosus Linnaeus 1753 changed with seasons in the Kieler Fjord, part of the Baltic Sea.

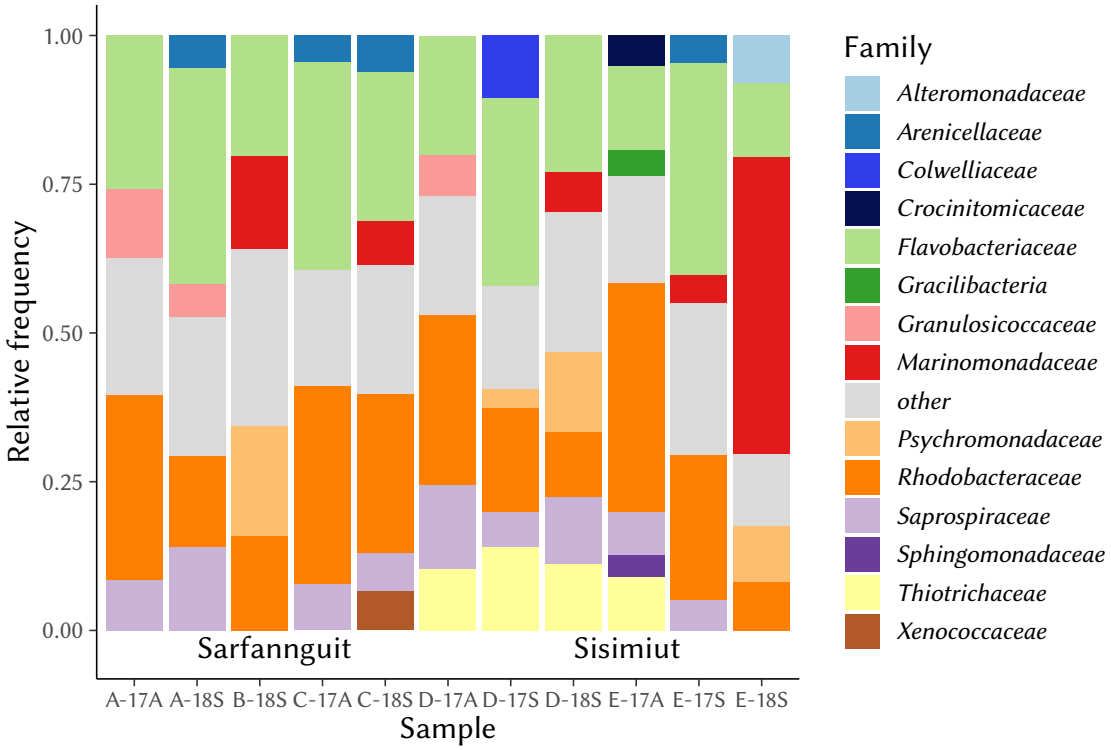


Figure 7.2: Relative abundance of surface bacterial families across all samples of *Fucus* sp. from Greenland. Samples were from Sarfannguit (A, B and C) and Sisimiut (D and E), collected in 2017 (17) and 2018 (18), in August (A) and September (S). The category "other" contains all classes with less than 2000 reads for the individual sample, which was also the case for all unassigned reads.

Flavobacteriaceae contain over 90 genera and hundreds of species, amongst them fish pathogens such as *Flavobacterium psychrophilum* and human pathogens [141]. *Rhodobacteraceae* are aquatic

bacteria frequently found in marine environments, involved in the biogeochemical cycles of sulphur and carbon, and in symbiosis with microorganisms and macroorganisms [179]. Most members of the family *Thiotrichaceae* are associated with aquatic environments and deposit sulphur [83]. *Thiotrix*, a genus in the family of *Thiotrichaceae*, has been found in connection with sulphide containing waters, and in activated sludge systems for the treatment of septic or sulphide-bearing wastewaters [83].

Two of the most common families (*Flavobacteriaceae* and *Rhodobacteraceae*) in this study were also identified amongst the three most common families on another macroalgae, *A. nodosum*, which is also in the Fucaceae family [140].

Beta diversity Comparative analysis of the microbiome samples via principal coordinate analysis (PCoA, figures 7.3, page 89 and 7.4, page 90) revealed three clusters: Sarfannguit, Sisimiut site D and Sisimiut site E.

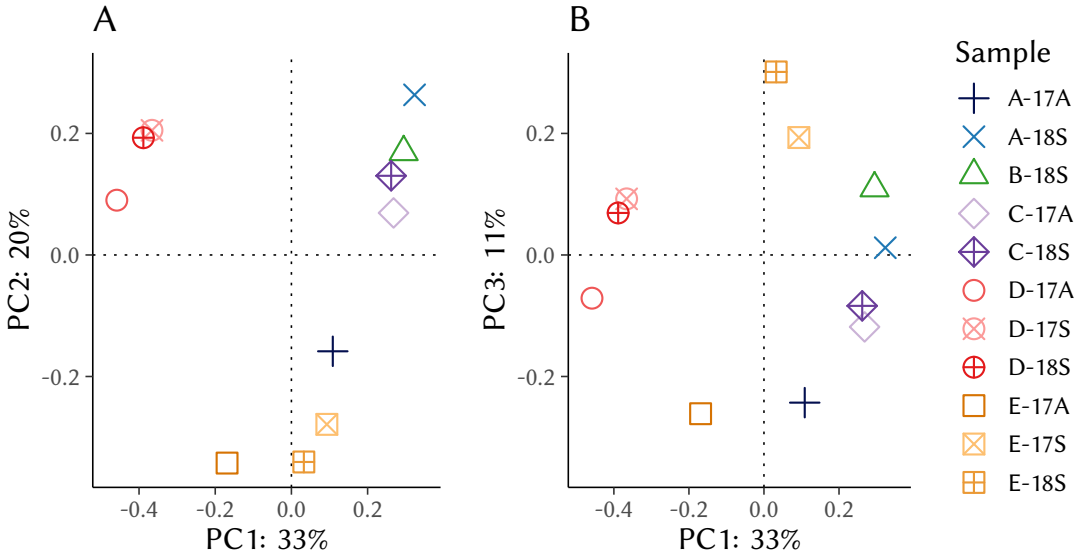


Figure 7.3: Jaccard distance based PCoA plot of microbiota from *Fucus* sp. from Greenland. Samples were from Sarfannguit (A, B and C) and Sisimiut (D and E), collected in 2017 (17) and 2018 (18), in August (A) and September (S). Panel A) PC1 and PC2; panel B) PC1 and PC3.

The August 2017 sample from site Sarfannguit A (A-17A) is a clear outlier from the other Sarfannguit samples, with a microbiome much more resembling the Sisimiut site E microbiomes. Factors influencing this separation could be the number of households discharging to the respective locations. Sisimiut site E receives effluent from the regional hospital, which also receives patients from surrounding communities, which could influence the microbiome.

The difference between years is not very pronounced, except in the case of the Sarfannguit site A (A-17A), which is associated with all three samples from the Sisimiut site E (E-17A, E-17S and E-18S) along the first two principal components (PC1 and PC2), both for Jaccard distance (see figure 7.3, page 89, panel A) and unweighted unifrac (see figure 7.4, page 90, panel A). The weather was warm during the sampling period in August 2017, with day air temperatures up to 20 ℃, which may have contributed to a different environment for the microbiome in the surface waters.

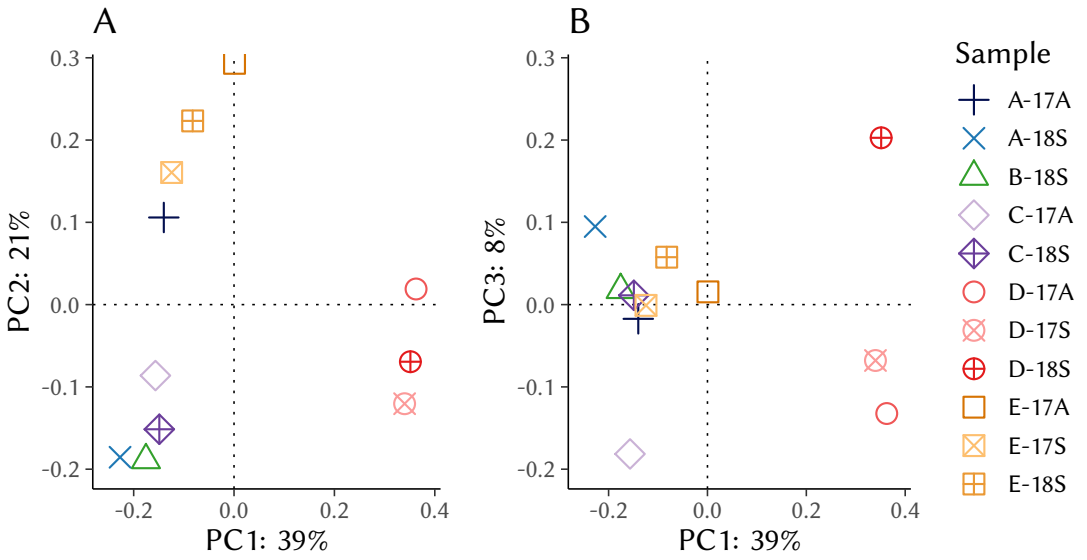


Figure 7.4: Unweighted unifrac based PCoA plot of microbiota from *Fucus* sp. from Greenland. Samples were from Sarfannguit (A, B and C) and Sisimiut (D and E), collected in 2017 (17) and 2018 (18), in August (A) and September (S). Panel A) PC1 and PC2; panel B) PC1 and PC3.

7.4.4 Legislation of microbial food safety of fresh seaweed

While seaweed is not explicitly named in the European consolidated commission regulation No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, [66] or Danish legislation concerning microbial criteria, France has set out the following rules which apply for dried seaweed product: mesophilic aerobic bacteria < 100 000 g^{-1}, faecal coliforms < 10 g^{-1} and Salmonella absence in 25 g of dried product [78]. If the same rules were to apply to fresh seaweed, as studied here, all samples from Sisimiut would fail these microbial criteria due to the presence of E. coli and coliform bacteria.

The European Union is currently collecting information on the dietary exposure to some elements of concern in seaweed [65]. Unfortunately, there is no corresponding effort put into

the development of microbial food safety criteria.

7.5 Conclusion

In this study, seaweed (*Fucus* sp.) and seawater was collected from two locations (two presumed clean and one close to the wastewater outlet) in Sarfannguit, a smaller settlement, and two sites close to municipal wastewater outlets at Greenland's second biggest town, Sisimiut.

The microbial community of wild *Fucus* sp. was affected by wastewater emissions. Human pathogens and indicators of human faecal contamination were present on the seaweed at both locations in Sisimiut, but not in Sarfannguit. Bacterial counts differed between seaweeds and the surrounding seawater. Thus, seaweed should always be sampled directly when investigating contamination, because low concentrations in the seawater were not a universal predictor for low concentrations on the seaweed. *Alphaproteobacteria*, *Bacteroidia* and *Gammaproteobacteria* were the three most common classes of bacteria found, and the two major bacterial families were *Flavobacteriaceae* and *Rhodobacteraceae*.

In this study we documented the presence of human pathogens on seaweeds harvested at sewage exposed sites in Greenland. Therefore, we strongly recommend that there should be made rules on distance of seaweed harvesting from wastewater outlets and dump sites. Similar rules are already in place for fishery exclusive zones, for example in Sisimiut. We furthermore recommend that legislation should be put into place regarding microbial safety standards for seaweed.

7.6 Acknowledgements

We thank Mia Laursen for her help in the laboratory and Margrethe Carlsen for the MALDI-TOF MS analysis.

7.7 Author contributions

CRediT (Contributor Roles Taxonomy) author contributions
KJK - Conceptualisation, data curation, formal analysis, funding acquisition, investigation, project administration, visualisation, writing - original draft
JSS - Data curation, formal analysis, investigation, writing – review & editing
LTH - Conceptualisation, funding acquisition, project administration, resources, supervision, writing - review & editing
PEJ - Conceptualisation, funding acquisition, project administration, supervision, writing - review & editing

8 Kainic acid in *P. palmata*

8.1 Introduction

Kainic acid, a potent neurotoxin, was originally discovered in the red seaweed *Digenea simplex* (Wulfen) C.Agardh 1822 by Japanese researchers in the 1950ies. The predominant localisation of kainic acid in surface cells of *D. simplex* points towards a role in chemical defence [195]. Kainic acid has since been documented in several other seaweeds, which all belong to Rhodophyta. *Palmaria palmata*, dulse, has also been shown to contain kainic acid, as reported in four published studies [106, 124, 148, 182]. A 2011 report by the National Food Institute of the Technical University of Denmark lists kainic acid as a concern for *P. palmata*, and points to the lack of information on the concentration of the toxin in dulse from Denmark and abroad [169]. Subsequently, kainic acid has also been reported in Danish *P. palmata* by Jørgensen and Olesen [106]. However, there exist no established safe intake levels for kainic acids.

Recently, Chekan et al. [34] identified the kainic acid biosynthesis gene clusters in both *D. simplex* and *P. palmata*, and were able to prove the biosynthesis pathway from L-glutamic acid and dimethylallyl pyrophosphate via N-prenylation to prekainic acid, and subsequent cyclization to kainic acid (see figure 8.1). Chekan et al. [34] were also able to produce kainic acid in gram scale in vitro by expression of the biosynthetic genes in *Escherichia coli*.

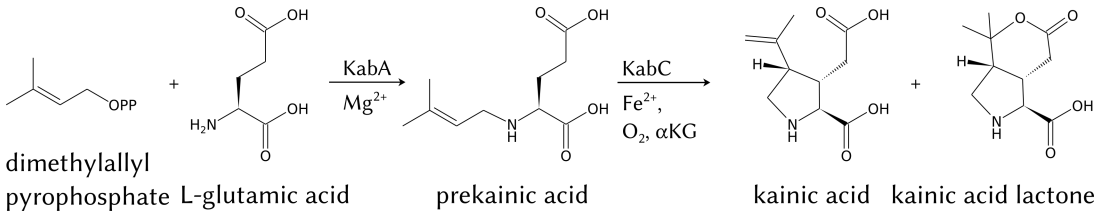


Figure 8.1: Kainic acid biosynthesis pathway as proposed by Chekan et al. [34]. Abbreviations: KabA N-prenyltransferase (KabA), KabC αKG-dependent dioxygenase (KabC), α-ketoglutarat (αKG). Redrawn from the original publication.

To obtain more knowledge about the concentration of kainic acid in Greenland seaweed, we investigated the content of kainic acid in both fresh and artisanally dried *P. palmata* harvested in three locations in Greenland.

8.2 Materials and methods

Samples of fresh *P. palmata* were collected in Sarfannguit in 2017 and Nuuk in 2018. Dried *P. palmata* was received from the Sisimiut area in 2017. The fresh *P. palmata* was frozen in Greenland, transported frozen to Denmark, freeze dried and pulverised, as described in previous sections (see chapter 5, section 5.3.2, page 29). The dried *P. palmata* was also pulverised but yielded a much coarser powder, due to its leathery consistency.

All laboratory analyses were carried out by the Research Group for Analytical Food Chemistry, National Food Institute, Technical University of Denmark, following the methodology

described in [106]. Briefly, 1 g of powder sample was extracted in a stepwise methanol/water extraction. Filtered extracts were quantified through liquid chromatography with tandem mass spectrometry (LC-MS/MS). A calibration curve was prepared from matrix-matched kainic acid from 280 ng mL^{-1} to 9,400 ng mL^{-1} extract, corresponding to 7 µg g^{-1} to 235 µg g^{-1} kainic acid in the seaweed. Samples with higher concentrations were diluted. Kainic acid was quantified based on the daughter ion m/z 122.

8.3 Results and discussion

Table 8.1 summarises the results from this study and other published studies on *P. palmata*. We found a factor ten difference between fresh and dried Greenlandic *P. palmata*, with a higher kainic acid concentration of 241 - 661 µg g^{-1} dry weight detected in the fresh seaweed compared to 33 - 55 µg g^{-1} dry weight in the dried seaweed. However, the observed range of kainic acid content in the dried and fresh materials is comparable to concentrations reported for both dried and fresh dulse in other studies (see table 8.1, page 96), therefore it cannot be concluded that the drying was responsible for the observed difference in dulse from Greenland. The highest documented concentration of kainic acid at 10,000 µg g^{-1} in dried seaweed was found in Canadian dwarf mutant strains [182]. In regular phenotypes, the highest documented concentration was 4,000 µg g^{-1} in fresh Canadian *P. palmata* [124]. The wide variability in kainic acid concentrations has been attributed to differential genetic expression by Ramsey et al. [182], and may be influenced by environmental factors.

There are no published studies on the bioavailability of kainic acid after oral intake of the pure substance or in a food item (search terms “kainic acid” AND ingestion; “kainic acid” AND bioavailability; search engines: DTU Findit, Google Scholar, PubMed, Scopus; date of search: 2020-11-20). All published studies focus on the injection of pure kainic acids into the brain or body cavity in rat or mice, to induce seizures, hippocampal damage, and behavioural changes. Even if these studies were fully translatable to kainic acid ingested by humans, Mouritsen et al. [148] estimate that they would translate to 2 g pure kainic acid for an adult human. This would require the consumption of roughly 3 kilograms of fresh dulse from Nuuk in one meal, which can be considered a highly unlikely event.

8.4 Conclusion

Kainic acid was detected in both fresh and dried samples of *P. palmata* from Sarfannguit, Sisimiut and Nuuk. There are no scientific studies available on the bioavailability of kainic acid after ingestion, and no safe intake levels for kainic acids have been established. However, to obtain concentrations comparable to the amounts injected into rodents for the induction of seizures, improbable amounts of fresh *P. palmata* would need to be consumed. The detected levels of kainic acid in Greenland dulse are therefore unlikely to constitute a chemical hazard that would prevent human consumption of this seaweed resource.

Table 8.1: Kainic acid (µg g^{-1} dry weight) in fresh and dried *P. palmata* from Greenland and other locations from published literature.

Country	Location	Dried/fresh	Kainic acid (µg g^{-1} dw)	Literature source
Greenland	Nuuk	fresh	661	This study; sampleID 90, powderID 77
	Sarfannguit	fresh	241	This study; sampleID 95, powderID 82
	Sisimiut	dried	55	This study; sampleID 56, powderID 49
	Sisimiut	dried	33	This study; sampleID 57, powderID 50
Canada	Bay of Fundy (Minas Basin)	dried	< 4000	Ramsey et al. [182]
	Grand Manan Island, Charlotte County, New Brunswick	dried	>10000*	Ramsey et al. [182]
	Halifax County, Nova Scotia	fresh	4000	Laycock, Freitas, and Wright [124]
	South Wolf Island, Charlotte County, New Brunswick	dried	>10000*	Ramsey et al. [182]
China		dried	< 0.7 (LOQ**)	Jørgensen and Olesen [106]
Denmark		fresh	0.1	Mouritsen et al. [148]
		fresh	< 0.7 (LOQ) up to 560	Jørgensen and Olesen [106]
France		dried	7-100** up to 470	Jørgensen and Olesen [106]
Iceland		dried	21	Mouritsen et al. [148]
		dried	< 0.7 (LOQ) up to 15	Jørgensen and Olesen [106]
Ireland		dried	< 4000	Ramsey et al. [182]
		dried	< 0.7 (LOQ) up to 270	Jørgensen and Olesen [106]
Norway		dried	240	Jørgensen and Olesen [106]
Spain		dried	18	Jørgensen and Olesen [106]
USA	Maine	fresh	0.22	Mouritsen et al. [148]

*Dwarf mutant strain that had been kept under laboratory conditions and may have been contaminated by epiphytes. ** Limit of quantification (LOQ). *** 7-100 µg/ g dry weight was the concentration range given in the publication.

8.5 Acknowledgements

Kevin Jørgensen is thanked for analysing the Greenlandic *P. palmata* samples.

9 Shelf-life of washed or blanched Danish sugar kelp

Publication information

This chapter reports partial results of a larger study which was carried out in collaboration with Cecilie Wirenfeldt Nielsen and Jonas Steenholdt Sørensen.

9.1 Introduction

Seaweed is an up-and-coming food item in the Nordic countries and Europe in general [14] and may be purchased fresh from some vendors [45]. To ensure good hygiene of foodstuffs, washing is a crucial step both during production and at the final consumer. In the European Union, and thus also Denmark, food safety legislation demands that food items need to be washed in clean water, specifically mainly potable water [70]. Whole fishery products and live bivalve molluscs, echinoderms, tunicates, and marine gastropods may, however, be washed in clean seawater [70]. Currently, no rules exist specifically for seaweeds, and authorities variably interpret them as being whole fishery products, allowing the use of clean seawater, or view seaweed as a harvested vegetable commodity for which the use of potable water is required. Even within a country as small as Denmark, some companies are obliged to wash their seaweed in potable water. This leads to some operators having to use heavily salted potable water, at great expense to the company, to achieve the desired quality (S. L. Holdt, personal communication, March 3rd, 2020).

Washing seaweed in clean seawater would be an economically and environmentally friendly alternative. Furthermore, producers report that washing seaweed in potable water accelerates its degradation (U. Lyberth, personal communication, August 9th, 2017). Very few studies exist on the shelf-life of fresh seaweed in general, or specifically on the effect that washing in potable compared to seawater has on the shelf-life of fresh seaweed. Liot, Colin, and Mabeau [130] investigated the microbiology and shelf-life of unwashed as well as seawater and freshwater washed dulse (*Palmaria palmata* Weber & Mohr 1805) and sea lettuce (Ulva rigida C. Agardh 1823). They found that seaweed washed in potable water degraded in less than seven days, while seaweed washed in seawater and freshwater stayed acceptable for 14 days [130].

Nayyar [158], who investigated the shelf-life of sugar kelp (*Saccharina latissima* (Linnaeus) C.E. Lane & C. Mayes Druel & G.W. Saunders 2006) at different storage temperatures and harvesting times, reported drops in sensory evaluation scores on day 7 for a February harvest, and on day 10 for a June harvest. However, they did not investigate sensory parameters between day 3 and 7 for the February harvest, and day 8 and 9 for the June harvest. Therefore, their observations can only be interpreted as minimum 3 and 8 days, respectively, of shelf-life. Also, they did not investigate the effect of washing in potable compared to seawater.

Some kelp species, such as the sugar kelp (S. latissima) used in this study, may have an undesirably high iodine content [114, 161]. Blanching has been identified as an effective method to decrease the iodine content in sugar kelp [161]. Blanching has also been found to increase

Table 9.1: Treatments and codes for the washed and blanched Danish sugar kelp *Saccharina latissima*.

Code	Description	Processing
HS	As harvested	No processing prior to packaging
WS	Washed seawater	Washing in seawater for 5 min at 15.5 ℃
WF	Washed potable water	Washing in potable water for 5 min at 4.0 ℃
BS	Blanched seawater	Blanching in seawater for 2 min at 80 ℃ and cooling after blanching at 4.2 ℃
BF	Blanched potable water	Blanching in potable water for 2 min at 80 ℃ and cooling after blanching at 15.5 ℃

the content of some valuable compounds such as the protein quality and content of polyunsaturated fatty acids [161] and may be an interesting tool to produce new food items with diversified visual and organoleptic properties. However, no studies exist examining the effect of blanching in potable compared to seawater, and the effect of blanching on the shelf-life of seaweed, and specifically sugar kelp.

We therefore addressed these gaps in knowledge in the current study, where our objective was to determine the shelf-life of fresh sugar kelp 1) washed in potable water, 2) washed in seawater, 3) blanched in potable water, 4) blanched in seawater and 5) as harvested (control).

9.2 Materials and methods

9.2.1 Sample collection

Sugar kelp (*S. latissima*) was harvested from a commercial cultivation site in Rørvig, Denmark (N55°56' and E11°46') in May 2020, at a salinity of 22 psu. The harvest was after 7 months of sea cultivation, and blades were cut by hand just above the growth zone (around 10 to 15 cm from the stem, which was left behind together with the holdfast), resulting in around 80 cm of blade per specimen. The seaweed was immediately brought to the laboratory and transported in food grade plastic containers at an average temperature of 16 ± 5 ℃ for 2.5 hours. At the laboratory, the seaweed was stored at 3 ℃ until further processing on the same day.

9.2.2 Sample processing

The seaweed was randomly divided into five batches and processed by washing, blanching or no processing, as detailed in table 9.1. Both washing and blanching was carried out in batches, and after either washing or blanching, the seaweed was left to drip off in trays for three to four minutes.

After the seaweed had dripped off, aliquots of 50 ± 5 g seaweed, corresponding to one to four individuals, were packed into a large weighing boat, and sealed in 70 µm polyethylene plastic bags with high permeability of >6 g m^{-2} d^{-1} for water vapour, >3 000 cm^3 m^{-2} d^{-1} atm^{-1} for O_2 and >14 000 cm^3 m^{-2} d^{-1} atm^{-1} for CO_2 (H902, Topiplast A/S, Greve, Denmark) using a Multivac C500 packaging machine (Multivac A/S, Vejle, Denmark).

Five bags per processing treatment were packaged separately for the respiration study in 117 ± 6 µm laminate film with low gas permeability of 0.45 cm^3 m^{-2} d^{-1} atm^{-1} for O_2 and 1.8 cm^3 m^{-2} d^{-1} atm^{-1} for CO_2 (NEN 40 HOB/LLPDE 75, Amcore, Horsens, Denmark).

The samples were stored in a cooling room at 2.8 °C ± 0.4 °C until analysis. Samples were analysed on day 1, 3, 5, 7, 9, 13 and 16.

9.2.3 Culture dependent microbiology

All culture dependent microbiology assays were carried out in triplicate from separate bags for each treatment and sampling day. Fifteen grams of seaweed were randomly cut into smaller pieces and mixed with 135 g chilled physiological saline with 0.1 % peptone. The subsequent treatment and serial tenfold dilutions were carried out as described in detail by Sørensen [214]. All agars were prepared according to manufacturer's instructions unless reference to Sørensen [214] is made.

For the quantification of total aerobic viable counts, marine agar was used [172] and incubated at 15 °C for seven days, as previously reported by Broekaert et al. [30]. To enumerate *Pseudomonas* spp., CFC was used [214], for H_2S producing bacteria (Shewanella spp. produces black colonies) iron agar [214], for actinomycetes actinomycete isolation agar (AIA) [143] and for yeasts and moulds oxytetracycline-glucose-yeast extract agar (OGYE) [225]. Colony forming unit (CFU) counts are reported.

Surplus material from the bags was used to determine water activity on the same day or stored at −20 °C until determination of pH.

9.2.4 Respiration

The respiration rate of the seaweed was measured as an indicator for its deterioration, as higher respiration rates have been shown to relate to shorter shelf-life in harvested fruit and vegetables [231]. Headspace gas composition (CO_2, O_2 and rest gas) for five replicates of each treatment was determined twice per sampling day.

9.2.5 Sensory evaluation

To quantify the intensity of the investigated sensory attributes, a descriptive profile attribute analysis was conducted. A panel of 4 to 5 judges evaluated the sensory profile on day 1, 3, 5, 7, 9, 13, and 16, coordinated by a panel leader who did not participate in the evaluation of the sensory profile but was responsible for the sample setup, blinding and recording. The line scale was 15 cm long with two anchors 2 cm from each end. The evaluation was conducted

in Danish, the mother tongue of all panellists, and the attribute descriptions later translated to English, see list below. The original evaluation sheet is attached in appendix B (page 160).

Sensory evaluation attributes

Visual appearance

- Firm (freshly harvested sugar kelp has a nice, firm consistency and does not lie flat)
- Transparent
- Uniform colour (low scores were given for spots or blotches)

Odour

- Beach cast (Odour of seashore on a warm summer day, or hay like)
- Boiled peas (Green sweet, cloying)
- Fresh sea
- Pleasantly sour
- Rubber
- Sweet
- Umami

Texture

- Leather (Low values represent tough stretchy material, higher values more brittle)
- Silky
- Slimy

The attributes were decided in consensus by the panel on the first day of analysis. For this, the 1-day old and some 5-day old, processed seaweed were analysed. Due to the harvest setup, it was not possible to include 16-day old seaweed. Three replicates of each treatment were blind labelled by the panel leader with a three-digit code and randomly arranged for analysis. Samples were kept on cooling plates with moist clean linens on top to prevent changes to the sensory evaluation due to the length of the evaluation procedure. Samples were assessed individually under artificial daylight (6 500 K, L 36W 965 Lumilux De Luxe, Osram, Germany) and thereafter, a consensus was recorded by the panel leader. New characteristics and odours were also recorded.

9.2.6 pH

The pH was measured in three samples per treatment by stirring 5 g of seaweed in 25 mL distilled water for 1 hour and measuring the sample solution with an PHC101 probe (HACH COMPANY, Loveland, USA). Samples were measured at day 1 and day 13 of storage, and each sample was measured twice.

9.2.7 Water activity

Water activity (a_w) was determined from material from the same bag that was used for culture dependent microbiology. Two grams of seaweed were placed in sampling cups, tempered to 25 ℃ and measured on a water activity meter (Aqua Lab model 4TE, Decagon devices Inc., Pullman, US).

9.2.8 Statistical analyses

Data was analysed in and visualised with R version 3.4.4 (2018-03-15) [181] in RStudio version 1.1.463 [193] on a x86_64-pc-linux-gnu (64-bit) platform, with the packages: readxl [242], dplyr [243], stats [181], utils [181], and ggplot2 [241]. Data was assessed for normality with the Shapiro-Wilk test and homogeneity was tested with the Bartlett Test of Homogeneity of Variances. When preconditions for parametric tests were absent, differences between treatments were compared with the non-parametric Kruskal-Wallis test. Analysis of variance (ANOVA) was carried out with the aov function, and post-hoc analysis with Tukey's test with the TukeyHSD function. The confidence level was 95 % unless otherwise noted.

9.3 Results and discussion

9.3.1 Culture dependent microbiology

Total aerobic viable counts and *Pseudomonas* spp. were statistically significantly ($p<0.05$) reduced by the blanching treatments to levels of 2 log CFU g^{-1} respectively 0 log CFU g^{-1} (see figure 9.1, page 103 and table 9.2, page 105). However, from day 5, the growth on seawater blanched (BS) samples reached the same level (more than 5 log CFU g^{-1}) as observed for non-blanched samples, and from day 7 often exceeded that of non-blanched samples. For freshwater blanched samples (BF), the CFU counts were lower until day 13 (total aerobic viable count) and day 7 (*Pseudomonas* spp.). Liot, Colin, and Mabeau [130] reported mesophilic aerobe counts of 3 to 5 log CFU g^{-1} throughout their 14-day storage experiment. However, samples washed in potable water showed considerable growth over the first seven days with subsequent rapid deterioration in their study.

Nayyar [158] reported aerobic plate counts of 2 to 3 log CFU g^{-1} on day 1 rising to 3 to 4 log CFU g^{-1} at the end of storage (day 7 to 12). However, they used Petri film aerobic count plates, which in contrast to the marine agar used in this study, are not optimal for the growth

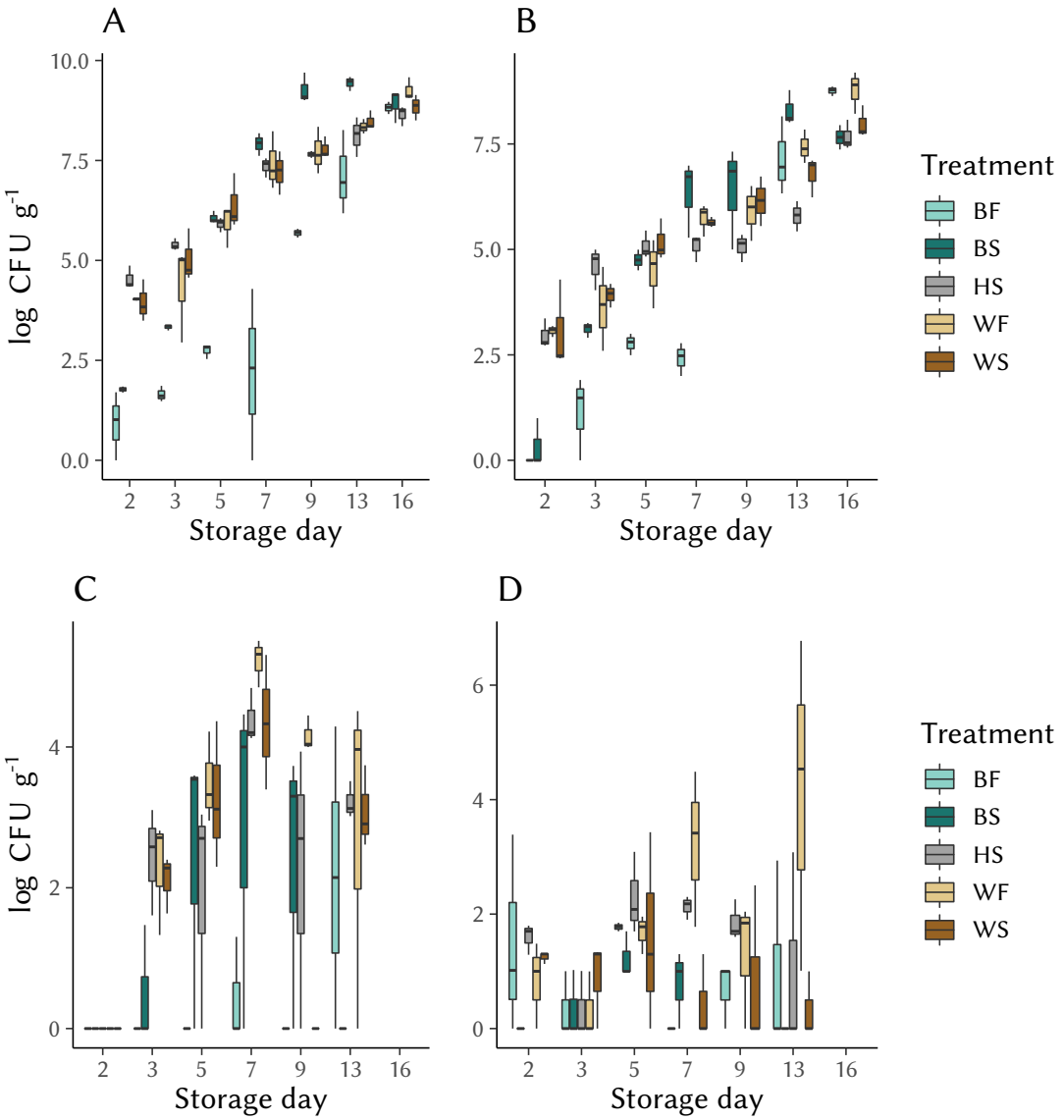


Figure 9.1: Colony forming unit (log CFU g^{-1}) counts of total aerobic viable counts (panel A), *Pseudomonas* spp. (panel B), *Shewanella* spp. (panel C) and actinomycetes (panel D) on washed or blanched Danish sugar kelp stored for 16 days. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range.

of marine bacteria (data not shown here). The high growth rates of *Pseudomonas* spp. observed in this study mean that these major spoilage bacteria for meat, poultry, milk, fish, and eggs [49] are also important actors for fresh and blanched sugar kelp.

Shewanella spp. were detected from storage day 3 onward in non-blanched samples at medians of 2.3 log CFU g^{-1} (WS) to 2.8 log CFU g^{-1} (WF), and to a lesser extent in BS (see figure 9.1, page 103 and table 9.2, page 105). At day 7, median *Shewanella* spp. concentrations topped at over 4 log CFU g^{-1} , and then dropped to between 2 to 4 log CFU g^{-1}. For the freshwater blanched samples, *Shewanella* spp. could only be detected after day 7 and day 13. The latter means that these bacteria were not evenly distributed throughout the seaweed material. *Shewanella* spp., an important group of fish spoilage bacteria [197], may therefore also be relevant spoilage bacteria for sugar kelp.

For actinomycetes, no discernible difference was evident between blanched and non-blanched samples (see figure 9.1, page 103 and table 9.2, page 105). Counts were below 2 log CFU g^{-1} on day 2 and colonies could not be detected in most samples except for WS at day 3. CFU counts increased to between 1 and 2 log log CFU g^{-1} on day 5. From day 7, WF samples had the highest counts of up to 4.5 log CFU g^{-1}. Actinomycetes are associated with taste and odour problems in potable water [171], but based on the results from this study, may not pose an issue for fresh and blanched sugar kelp.

Yeasts and moulds were statistically significantly reduced by the blanching treatment for the first five days of storage (see figure 9.2, page 104 and table 9.2, page 105). Colony forming unit counts were in the region of 1 to 4 log CFU g^{-1} throughout the storage period, except for 5.2 log CFU g^{-1} for WS at day 13.

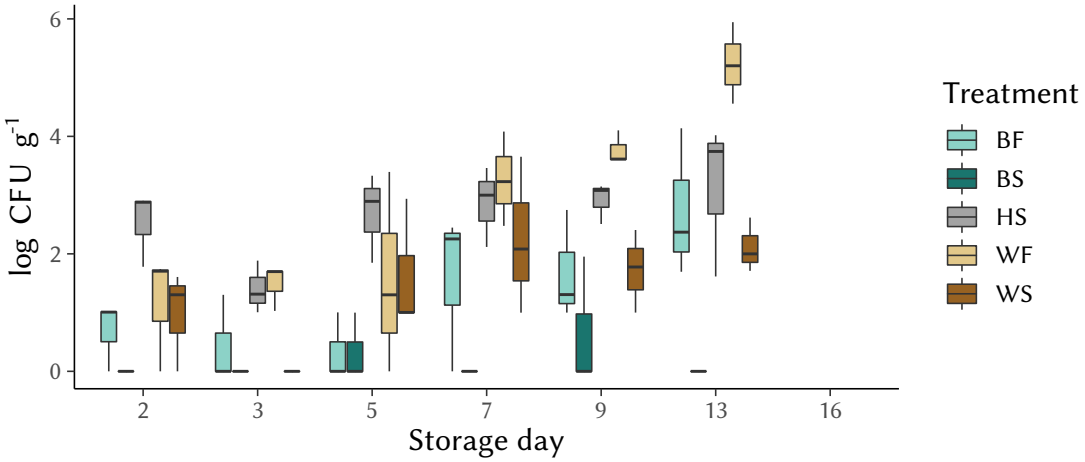


Figure 9.2: Colony forming unit (log CFU g^{-1}) counts of yeasts and moulds on washed or blanched Danish sugar kelp stored for 16 days. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range.

Thus, our study is both more and less sensitive than that of Liot, Colin, and Mabeau [130], who reported yeast concentrations of up to 4 log CFU g^{-1}, while fungi remained below detection limit (2 CFU g^{-1}) for non-washed and seawater washed seaweed throughout their 14-day storage experiment.

Analysis of variance (ANOVA) of the growth on different culture media revealed that both storage date and treatment had a statistically significant effect, see table 9.2 (page 105). Their interaction (expressed as storage day:treatment) was also statistically significant. Increased storage time generally led to higher CFU counts. Blanching generally led to an initial decrease in the CFU count.

Table 9.2: Results of the linear model (p-values) of different culture media for the microbial growth on different agar types from bacteria of washed or blanched Danish sugar kelp stored for 16 days.

Organism	Storage day	Treatment	Storage day:treatment
Total aerobic viable count	<2e-16 ***	<2e-16 ***	2.04e-12 ***
Pseudomonas spp.	<2e-16 ***	1.95e-14 ***	9.56e-13 ***
Shewanella spp.	1.69e-09 ***	1.65e-07 ***	0.00817 **
Actinomycetes	0.044156 *	0.000911 ***	0.023681 *
Yeasts and moulds	3.43e-08 ***	1.28e-13 ***	0.0172 *

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

9.3.2 Respiration

The concentrations of both oxygen (O_2) and carbon dioxide (CO_2) showed statistically significant differences between treatments and over time ($p < 0.01$). Blanching in freshwater (BF) led to almost stable oxygen and carbon dioxide concentrations throughout the whole storage period (see figure 9.3 page 106). This means there were next to no processes taking place requiring oxygen. Blanching in seawater (BS) only led to measurable CO_2 production (median concentration of 0.2 %) from day 6 onwards. This is paralleling the growth in total aerobic viable counts and *Pseudomonas* spp. as shown in figure 9.1 (page 103).

Both the as harvested samples (HS) and those washed in freshwater (WF) showed remarkably similar trends over time with oxygen decreasing (from median 19 % to 18 %) at the same time as carbon dioxide increased (from 0.8 % to 2 %) during the first five days, with a stable period until storage day eleven where a second decrease in oxygen took place simultaneously with an increase in carbon dioxide (final concentrations were 16 % O_2 and 4 % CO_2). Again, these developments in gas concentrations paralleled the growth in total aerobic viable counts and *Pseudomonas* spp. as shown in figure 9.1 (page 103).

Interestingly, the greatest initial decrease in oxygen and increase in carbon dioxide was observed for the samples washed in seawater (WS), which dropped from median 18 % to 16 % O_2,

while CO_2 increased from 1.6 % to 3.6 %. This trend inverted from day 4 onwards, and from day 7 onwards both the HS and WF samples showed steeper concentration changes. This increase in oxygen concentration and concurrent decrease points towards some process that creates oxygen. For WS, on day 16 both O_2 and CO_2 concentrations had again reached the same levels as on day 0.

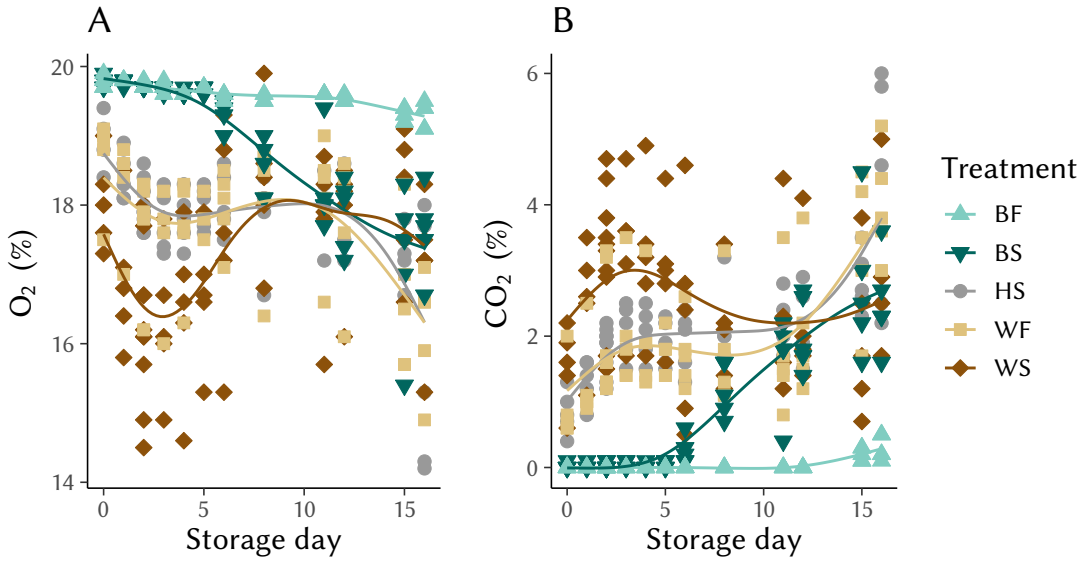


Figure 9.3: Oxygen (panel A) and carbon dioxide (panel B) concentration in closed containers used to store sugar kelp, pre-processed using five different treatments, for 16 days. Smoothed lines were calculated with a generalized additive model (GAM).

From figure 9.3 (page 106) it is also quite evident that there was a considerable spread in gas concentrations for a given treatment and storage day, which points towards differences in the processes taking place in the individual bags. The only exception from this was the samples blanched in freshwater (BF), where the gas concentrations were remarkably similar throughout the entire storage period. This issue of very differing gas compositions could have been mitigated by packing bigger samples, thus achieving a better approximation of the entire bacterial population on the sugar kelp. However, the investigated package size of around 50 g could be a good retail size, so our study is still a valid investigation.

9.3.3 Sensory evaluation

Visual attributes

The blanching treatment had a statistically significant effect on the "firm" attribute of the seaweed, which was lower for the blanched seaweed, as shown in figure 9.4, panel A (page 108), and table 9.3 (page 111). However, "firm" decreased for the washed and harvested treatments after day 7 and significantly dropped at day 16. "Transparency" (figure 9.4, panel B, page 108) was also affected by treatment and storage date, with "transparency" initially decreased in blanched and freshwater washed samples compared to the untreated samples (HS), but "transparency" was generally increasing over storage time for all treatments. No trend over storage time was discernible for "uniform colour" (figure 9.4, panel C, page 108).

Odour attributes

Odour attributes decreased over storage time, except for "beach" which only became noticeable from day 13 onwards (see figure 9.5, page 109, and panel A for "beach cast"). "Pleasantly sour" was stable for the first seven days of storage and dropped to a lower value from day 9 onwards (see panel D, figure 9.5 (page 109). The "boiled peas", "pleasantly sour" and "umami" odours were more pronounced in the blanched seaweed during the first five to seven days of storage (figure 9.5, page 109, panels B, D and G).

Texture attributes

There was no pattern evident for the "leather" attribute (see figure 9.6, page 110 and table 9.3, page 111). "Silken" texture was statistically significantly affected by the storage time, with increasing values from day 1 compared to later storage dates (except WF at day 16), but it was not statistically significantly affected by the treatment. "Slimy" texture was statistically significantly affected by the storage time, and the treatment, with a sharp increase in values from less than one to over 1 on storage day 9, this was especially evident for the BS and WF treatments.

Analysis of variance (ANOVA) of the different sensory attributes investigated revealed the storage day had a statistically significant effect on all attributes, except for "uniform colour" and "leathery", see table 9.3 (page 111). The treatment was only statistically significant for the visual appearance attributes, as well as four out of seven odour attributes ("beach cast", "pleasantly sour", "boiled peas", "umami") and the texture attribute "slimy". The interaction between storage date and treatment was statistically significant for "crisp", and five out of seven odour attributes ("rubber", "beach cast", "pleasantly sour", "boiled peas" and "umami"), but not for any of the texture attributes. This suggests that "leathery" is not suitable to detect storage day differences, or difference between treatments. Similarly, "uniform colour" is not suitable to assess storage date differences.

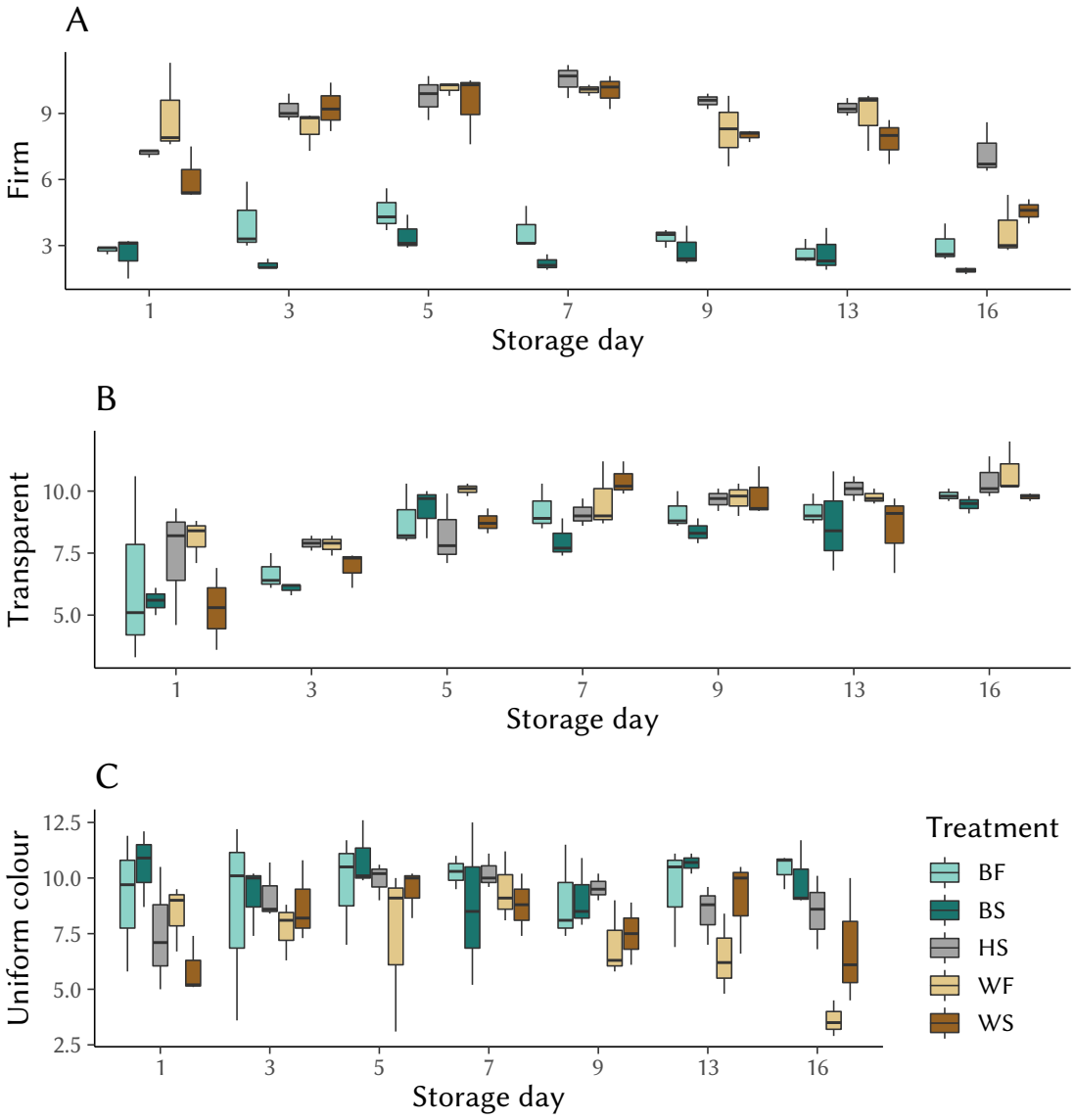


Figure 9.4: Visual sensory attributes for five different treatments of sugar kelp stored for 16 days. Panel A: Firm, B: transparent, C: uniform colour. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range.

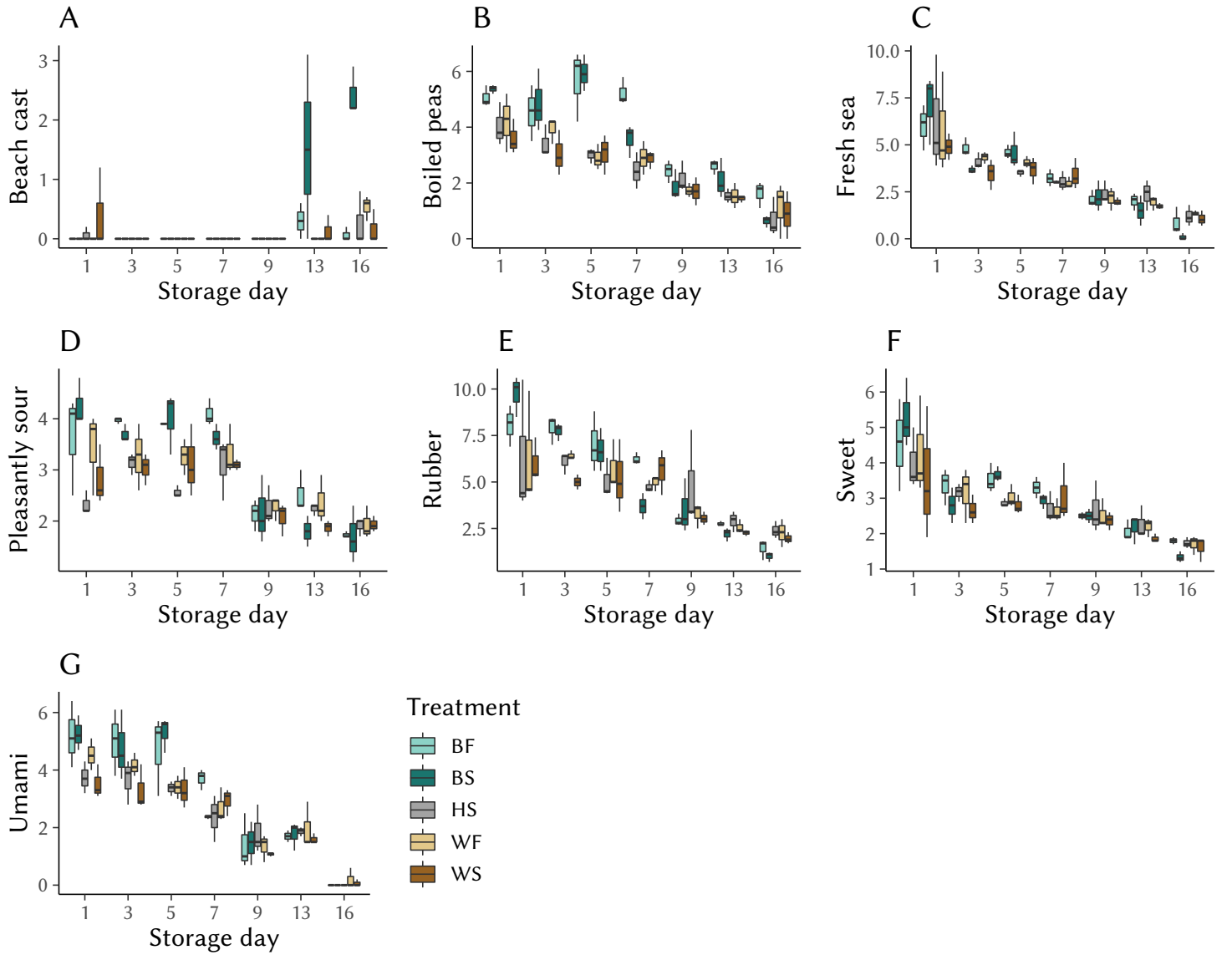


Figure 9.5: Odour sensory attributes for five different treatments of sugar kelp stored for 16 days. Panel A: Beach cast, B: boiled peas, C: fresh sea, D: pleasantly sour, E: rubber, F: sweet, G: umami. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range.

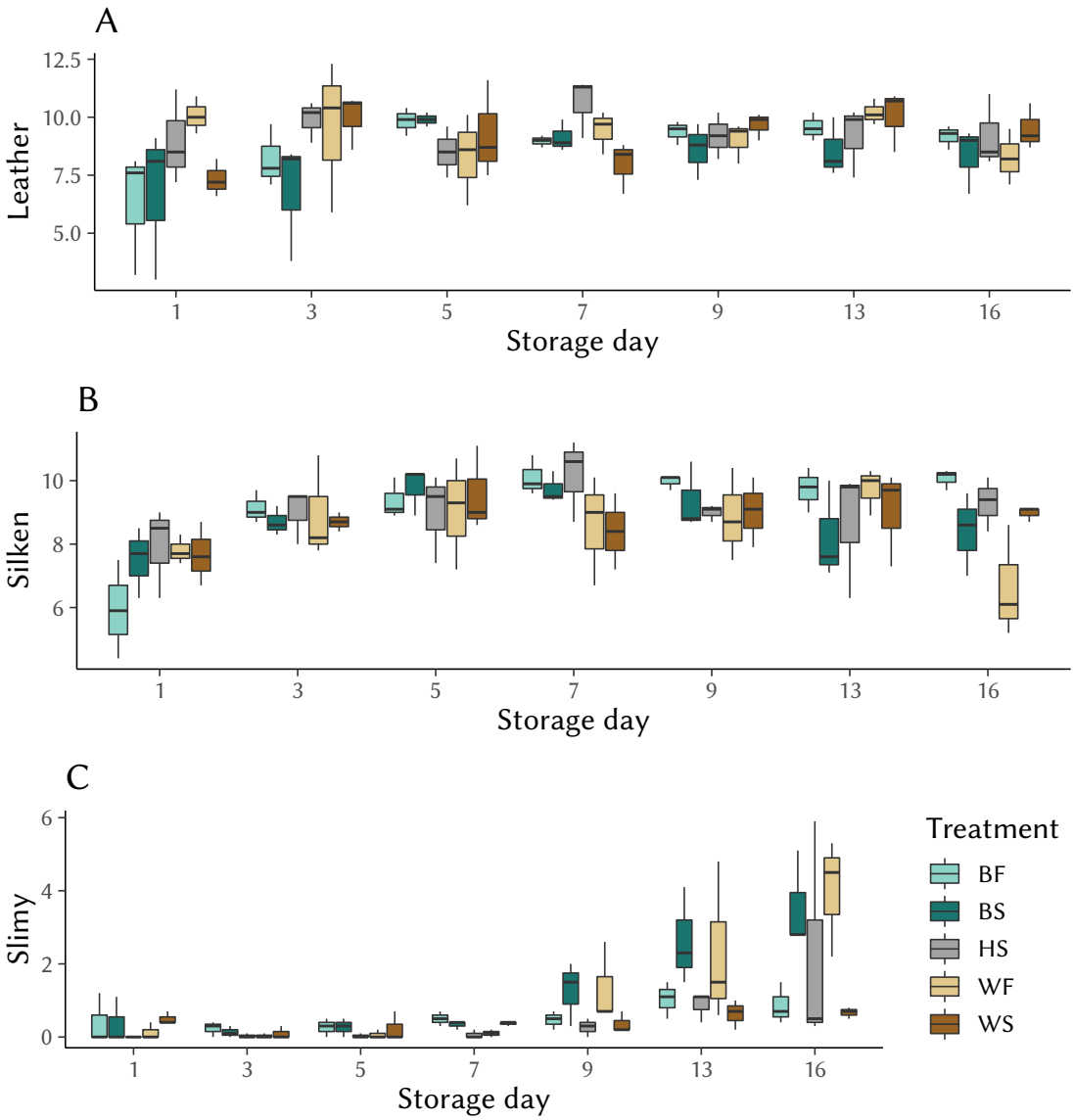


Figure 9.6: Texture sensory attributes for five different treatments of sugar kelp stored for 16 days. Panel A: Leather, B: silken, C: slimy. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range.

Table 9.3: Results of the linear model (p-values) fitted to sensory attributes of washed or blanched sugar kelp stored for 16 days.

Sensory attribute		Storage day	Treatment	Storage day:treatment
Visual appearance	Transparency	3.67e-13 ***	0.00121 **	0.71346
	Firm	2.59e-14 ***	<2e-16 ***	1.94e-05 ***
	Uniform colour	0.236973	0.000147 ***	0.458940
Odour	Sweet	<2e-16 ***	0.152	0.769
	Fresh sea	<2e-16 ***	0.526	0.749
	Rubber	<2e-16 ***	0.0751	0.0232 *
	Beach cast	1.43e-08 ***	1.25e-06 ***	2.28e-08 ***
	Pleasantly sour	<2e-16 ***	6.15e-06 ***	0.00577 **
	Boiled peas	<2e-16 ***	3.45e-12 ***	0.00188 **
	Umami	<2e-16 ***	0.000159 ***	0.035790 *
Texture	Leather	0.101	0.140	0.243
	Silky	6.87e-05 ***	0.401	0.207
	Slimy	2.82e-10 ***	0.00373 **	0.05141

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Qualitative odour descriptions

From storage day 9 onwards, negative odour descriptions such as "old flower vase water" and "rotten", "sour", "chlorine" became frequent. These could not be quantified with the experimental design used here but are a clear indication that the seaweed had reached the end of its shelf-life from a sensory point of view.

9.3.4 pH

The pH of the seaweed was statistically significantly elevated by the blanching treatment, from sour median pH values of around 6.2 to basic pH ranges of 7.7 for BS and 8.7 for BF (see figure 9.7, page 112). By storage day 13, the pH had dropped statistically significantly for all treatments, with blanched samples still significantly higher (median pH 6.8 for BS and 7.7 for BF) than those not blanched (pH around 5.7).

9.3.5 Water activity

Water activity was extremely high, in the region of 0.97 to over 0.99, see figure 9.8 (page 112). Blanching in freshwater (BF) resulted in consistently higher water activity, compared to the other treatments. Water activity levels stayed constant for the blanched samples throughout

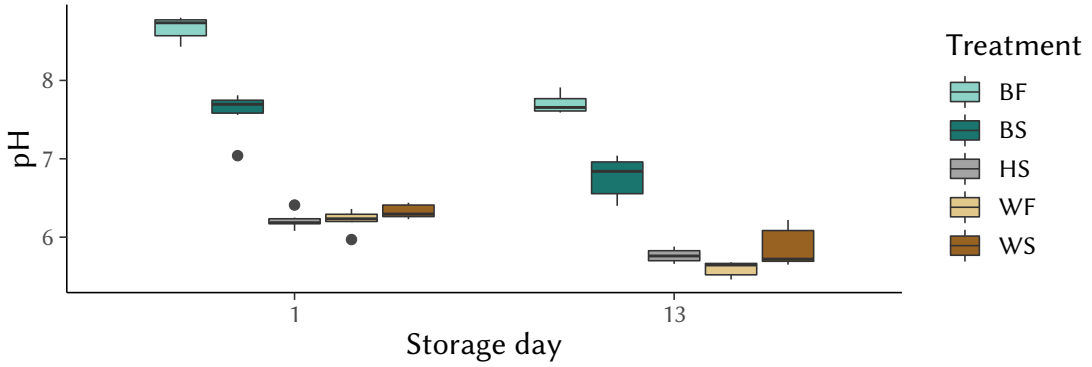


Figure 9.7: pH values for five different treatments of sugar kelp stored for 16 days. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range. Outliers beyond the whiskers are shown as circles.

the first nine days of storage, and then decreased. The same trend could be observed for the seaweed that had not been blanched, however, here the water activity decreased after day 7.

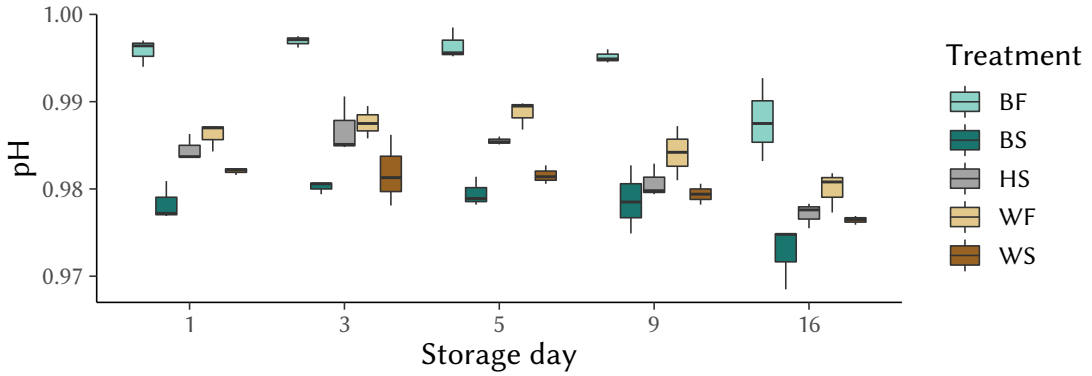


Figure 9.8: Water activity for five different treatments of sugar kelp stored for 16 days. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range.

The water activity range of non-blanched and blanched seaweed is comparable to that of fresh fruit and vegetables, which is in the region of 0.980 to 0.998 [36]. Silva et al. [207] reported similar water activities in the range of 0.970 for seaweeds from the Atlantic coastal waters of Bahia, in Brazil. Water activities in these high ranges mean that water availability is so high that there is no hindrance to mould, yeast, or bacterial growth and enzymatic activity is also

at its maximum [15].

9.4 Conclusion

Both *Pseudomonas* spp. and *Shewanella* spp. were important spoilage organisms as their numbers rose to coincide with sensory deterioration. In contrast, actinomycetes, yeast and moulds were of lesser importance due to their low numbers throughout the storage period.

The results from the culture dependent microbiology and the sensory evaluation as well as the respiration point towards a distinct shift happening after storage day 7, as observed by changed CFU counts and sensory attributes at storage day 9. Especially the negative odour descriptions recorded during the sensory evaluation suggest that consumers would reject the seaweed by storage day 9.

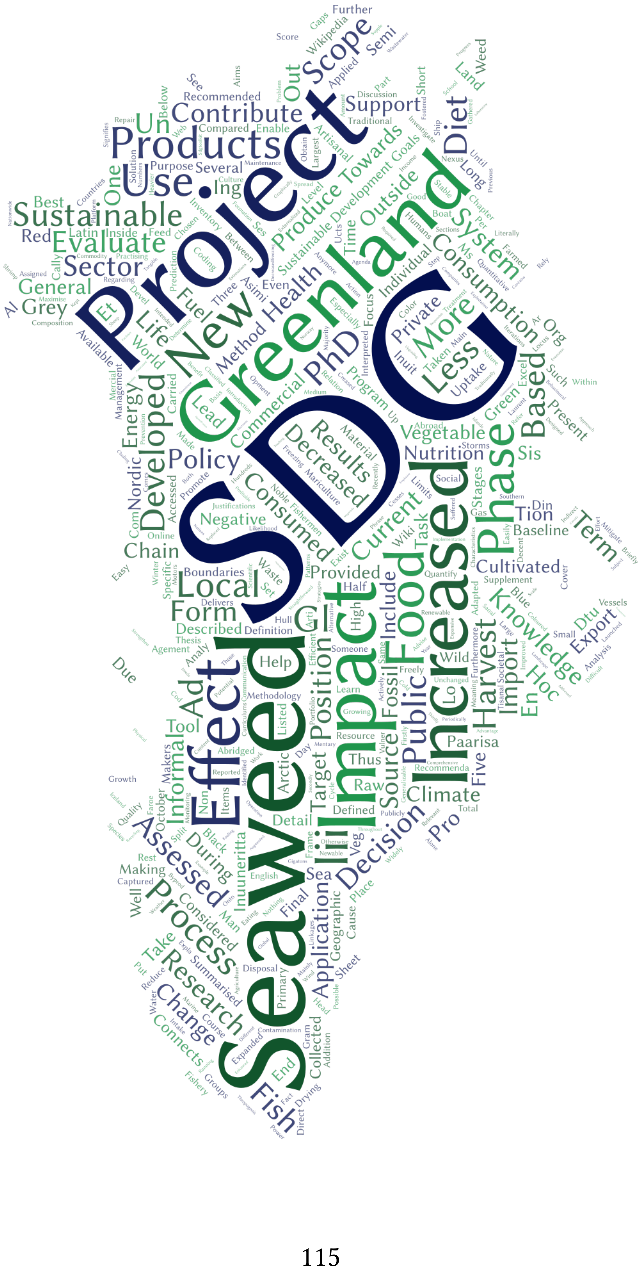
Shelf-life studies normally include a Weibull hazard analysis, where the end of shelf-life is determined as the point in time at which 50 % of consumers find the product unacceptable. Due to pandemic-related restrictions in Denmark in the summer of 2020, it was not possible to include a consumer panel in the present study. Future studies should therefore include consumer panels so a Weibull hazard analysis can be carried out to determine the shelf-life of fresh sugar kelp.

Blanching as a procedure to change the seaweed's sensory attributes was successful, especially odour attributes such as "boiled peas", "pleasantly sour" and "umami" were significantly increased compared to non-blanched samples. This opens interesting possibilities for product development.

9.5 Acknowledgements

This chapter reports partial results of a larger study which was carried out in collaboration with Cecilie Wirenfeldt Nielsen and Jonas Steenholdt Sørensen. They are thanked for their efforts with sample collection and lab work.

10 Impact on Sustainable Development Goals



10.1 Introduction

One of the most noble goals of research is to contribute to sustainable development. However, it is neither a straightforward, nor an easy task to quantify this contribution.

This chapter presents a semi-quantitative evaluation of the contribution to sustainable development of this PhD project. The contribution is evaluated in relation to the 17 UN Sustainable Development Goals (SDG) and their individual targets. This chapter is based on an analysis carried out during the October 2020 DTU PhD course 42750 Sustainability evaluation and communication.

10.2 Methodology

The UN SDG assessment methodology used is described in detail by Laurent et al. [122], and freely available in an abridged form [123]. This method was developed to enable a semi-quantitative evaluation of the contribution of research projects to sustainable development, and has been used to evaluate hundreds of PhD project at DTU since 2019.

Briefly, the method is based on comparing the world (described in a baseline system, where nothing has changed from the current day), as if the project did not exist, to the projected improved world (the new system, where the knowledge generated from the project has been applied).

The assessment is split into five phases. In phase 1, the application of the research project at societal level is defined. In phase 2, the scope of the assessment is defined. In phase 3, inventory is taken from the effects of the project application. In phase 4, the contribution of the applications to the SDGs are evaluated with the help of an MS Excel tool, and summarised graphically. Finally, in phase 5, the results of the assessment are interpreted and recommendations to mitigate negative impacts on SDGs are made. The assessment process includes several iterations, especially between phase 2 to phase 4.

Assessment method B was chosen, since this project can best be classified as one that delivers knowledge support for decision and policy making, which will lead to tangible decisions.

10.3 Results and discussion

10.3.1 Phase 1 - considered applications of the research project

The research project

Currently, most of the food consumed in Greenland is imported. Local products such as locally harvested and cultivated seaweed may contribute to a more sustainable food landscape. In addition, increased seaweed consumption may strengthen the position of the Greenland Inuit diet, because seaweeds have been consumed traditionally, even if they are not consumed that widely anymore. The PhD project had several aims, to cover current knowledge gaps: 1)

determine characteristics of Greenland seaweeds as food items, 2) investigate the impact of anthropogenic contamination in the form of wastewater 3) investigate the impact of processing on seaweeds.

Potential applications of the research project

For the purpose of this assessment, the geographical boundaries of Greenland were chosen as the limits, since the knowledge from this project is specifically relevant to Greenland. The time frame was set until 2030, mainly because long-term prediction is difficult for the food production sector in Greenland, which is currently actively developing towards more local production. Also, the the Sustainable Development Goals (SDGs), have a scope of 2030, and Greenland has recently launched its own homepage, monitoring progress towards the goals [5, 154]. Three target groups were identified for the knowledge generated in this PhD project: a) private individuals, b) the private sector (food producers) and c) the public sector (especially policy and decision makers). In Greenland, the knowledge collected in this project will lead to increased harvesting, production and consumption of local seaweed. School curriculums will include knowledge about seaweeds and their application, and seaweeds will be part of the Greenlandic Nutrition recommendations. Mariculture of local seaweed species will produce high quality (export) products, expanding the current portfolio of shrimp and fish products.

10.3.2 Phase 2 - Scope of the assessment

Definition of the baseline system

In the baseline system, all three target groups (individuals, the private and the public sector) obtain knowledge about nutritional composition of seaweeds from material gathered in other Arctic or Nordic countries, as well as informal knowledge[1] and ad hoc[2] commercial seaweed analysis. These information sources form the basis for decisions about collecting, farming and eating seaweed. Regarding vegetable consumption, the majority of vegetables consumed are imported from land-based agriculture abroad. A small amount of seaweed is harvested and consumed locally.

Definition of the new system

In the new system, the knowledge from this project is used to advise the public sector. This results in the uptake of seaweed into the public health program Inuuneritta III, running from 2020 to 2030. Furthermore, the nutritional content information is easily accessible and publicly

[1] Informal knowledge is information that has not been externalized or captured and the primary locus of the knowledge may be inside someone's head. Source: Wikipedia (https://en.wikipedia.org/wiki/Informal_learning, accessed 2020-10-24)

[2] Ad hoc is a Latin phrase meaning literally 'to this'. In English, it generally signifies a solution designed for a specific problem or task, non-generalizable, and not intended to be able to be adapted to other purposes. Source: Wikipedia (https://en.wikipedia.org/wiki/Ad_hoc, accessed 2020-10-24)

available (e.g. online at paarisa.gl[3] and asimi.gl[4]) The augmented knowledge about Greenland seaweeds results in increased consumption of seaweed, both from increased artisanal harvest (5 % to 20 % of fishermen have taken up seaweed fishing) of wild seaweed and the growing commercial seaweed cultivation sector (half or all of the large fishing companies are practising seaweed mariculture in 2030). Five to 20 % less imported farmed vegetables are consumed, because they have been replaced by Greenlandic seaweed din the diet. Greenland seaweed is produced as a high quality commodity for export. In figure 10.1 (page 118), those processes that take place outside the geographic boundaries of Greenland, and thus outside the scope of this assessment, are coloured in grey.

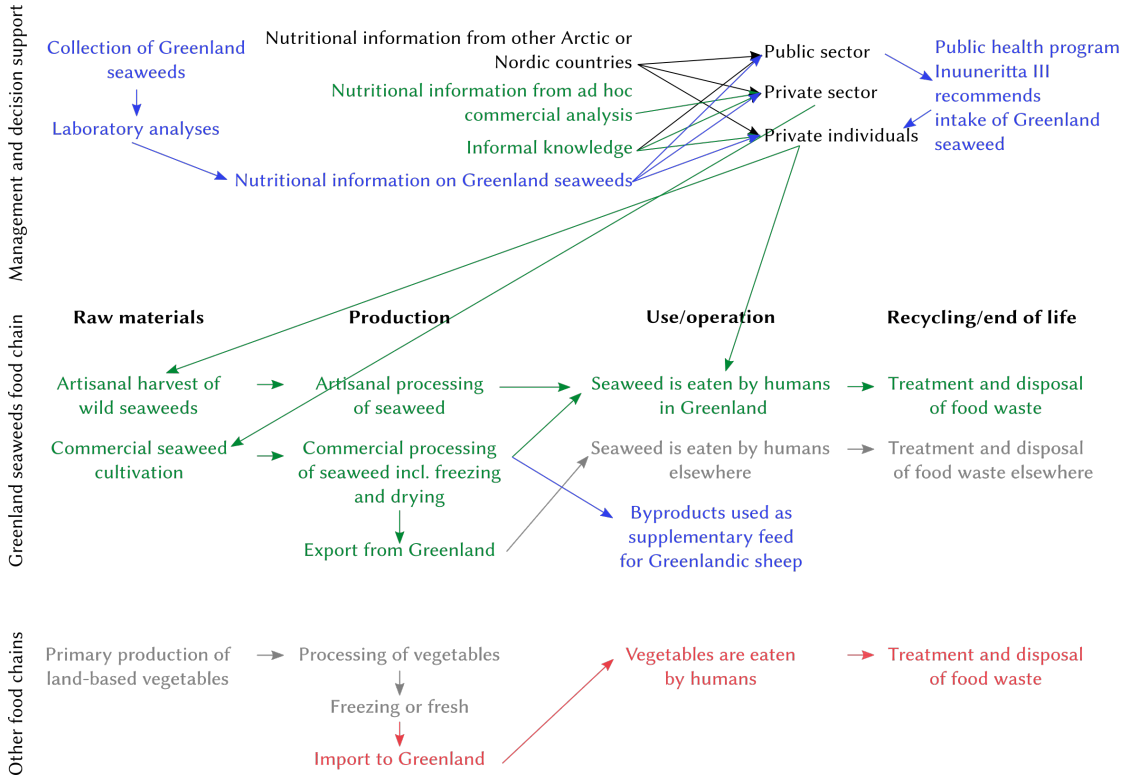


Figure 10.1: Impact of the knowledge from the research project on processes in management and decision support, the Greenlandic seaweeds food chain and other food chains. Color coding: black - unchanged processes, blue - new processes, green - increased processes, red - decreased processes, grey – processes outside the scope of the assessment.

[3]Paarisa is a program for health promotion and prevention within social and health, https://paarisa.gl/
[4]Asimi is a web based teaching resource on Greenland's nature, http://asimi.gl/

10.3.3 Phase 3 - Inventory of effects from the applications

The main direct and indirect effects of the application of the project are listed in table 10.1. Effects on processes that take place outside the scope of this assessment, i.e. outside Greenland, are listed in grey (see also 10.1 (page 118), but since they are not considered further, have not been assigned numbers.

Table 10.1: Impact of the knowledge from the research project on processes in management and decision support, the Greenlandic seaweeds food chain and other food chains. Color coding: black - unchanged processes, blue - new processes, green - increased processes, red - decreased processes, grey – processes outside the scope of the assessment.

Management, decision and policy support	Implementation of the decision and policy management support			
	Raw materials	Production	Use	Recycling & end of life
	Physical changes			
1 New collection of Greenland seaweeds and laboratory analyses during the project	7 Increased artisanal seaweed harvest	9 Increased artisanal processing of seaweeds	13 Increased consumption of Greenland seaweeds by humans	16 Increased treatment and disposal of seaweed food waste
2 Increased ad hoc commercial analysis	8 Increased commercial seaweed cultivation	10 Increased commercial processing of seaweeds including freezing and drying	14 New use of seaweed byproducts as supplementary feed for Greenlandic sheep	17 Decreased treatment and disposal of vegetable food waste
3 Increased informal knowledge	Decreased primary production of land-based vegetables	11 Increased export of seaweeds from Greenland	15 Decreased consumption of vegetables by humans in Greenland	
4 New nutritional information on Greenland seaweeds		Decreased processing of vegetables		
5 New recommendation on seaweed intake in public health program Inuuneritta III		Decreasedfreezing of vegetables		
6 Increased focus on producing and consuming locally produced food and traditional diets		12 Decreased vegetables import to Greenland		

The increased artisanal harvesting of wild seaweed (effect 7) can lead to a more stable income for fishermen who otherwise rely e.g. on cod fishery, which is subject to fish behavioural patterns, making the fishing periodically more or less profitable. Furthermore, Greenlandic fishermen have suffered from the effects of climate change in the form of less predictable weather and heavier winter storms, as for example reported from Southern Greenland [151]. Locally produced foods and the traditional Inuit diet in general will benefit from the increased focus on local and sustainable production and consumption fostered by the increased production and consumption of Greenlandic seaweed (effect 6).

10.3.4 Phase 4 - Evaluating the effect of the project on the SDGs

The electronic supplement to the thesis [115] contains the detailed results, summarised in figure 10.2 below. In the supplement, sheet "SDG_effect_linkages" connects the effects summarised in table 10.1 to the SDGs, their associated targets and justifications are provided. Connections between different SDG goals and targets were assessed with a nexus approach tool provided by sdgtoolkit.org [228]. Also in the supplement, sheet "SDG_Evaluation" provides estimations of the likelihood and magnitude of possible impacts on the SDGs. Justifications are provided as well as a quantification of the evaluation score.

10.3.5 Phase 5 - Interpretation

The largest impacts from this project are a negative impact on SDG 13 (Climate action), which is caused by the consumption of fossil fuels required for harvesting, processing and export of seaweeds. The largest positive impacts are connected to SDG 8 (Decent work and economic growth), SDG 12 (Responsible consumption) and SDG 14 (Life below water). SDG 8 and SDG 12 are positively impacted by the increased seaweed production. SDG 12 is furthermore positively impacted during the use and end-of-life stages, as seaweeds are more sustainable compared to imported foods, and cause less food waste. SDG 14 is positively impacted due to the enhanced scientific knowledge and sustainable exploitation of marine resources. SDG 13 is at the same time negatively and positively impacted by effects 7 and 8 (increased artisanal harvest and increased commercial cultivation). The positive impact is due to the fact that seaweed harvest and culture contributes to climate change adaptation, since seaweed is considered less vulnerable towards climate change than fisheries. The negative impact is due to the increased fossil consumption in connection with boat and ship operation.

This increased fossil fuel consumption during the raw materials, production and use stages needs to be addressed. At present, only 16 % of the total final energy consumption in Greenland comes from newable energy sources [5]. In the short and long term, several strategies should be considered to mitigate this increased fuel consumption. Firstly, vessels should be kept in good repair, which includes hull maintenance to reduce fouling and thus fuel consumption. As a medium term solution, upgrading to more energy efficient motors, or in the long term, energy efficient vessels are recommended. Secondly, basing commercial processing facilities

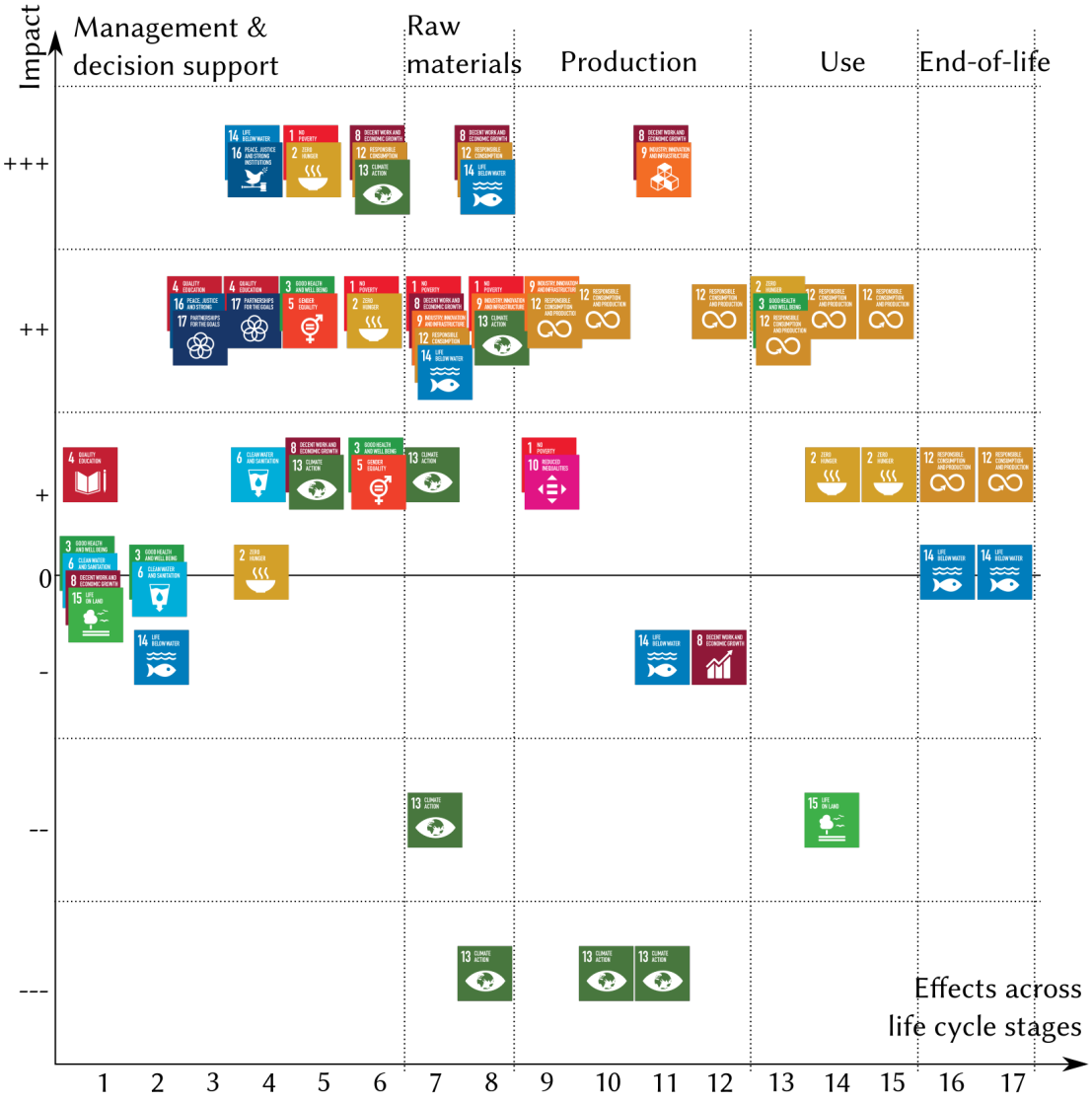


Figure 10.2: Effects on processes impacted by the PhD project across the life cycle stages and their impact on the SDGs. Refer to table 10.1, page 119 for explanations of effects 1 to 17.

where renewable energy sources such as hydropower or wind power are available. Thirdly, applied research into alternative methods for freezing and drying, taking advantage of the cold arctic climate should be carried out.

Other short term recommendations to maximise the positive impact of this project are to

emphasise knowledge support to decision and policy makers, so seaweed can be put onto the agenda of the public health program Inuuneritta III. Greenland has also presented its own SDG platform in 2020 [5, 154]. This effort from the government to promote the SDGs will help to spread the knowledge from this project to impact management and decision making processes described in the previous sections. Especially the uptake of seaweed into the public health program Inuuneritta III [152] will be an important step towards nationwide uptake of seaweed into diets.

This assessment was limited to the geographic boundaries of Greenland. The assessment could be expanded to include the effects of increased seaweed production and consumption throughout the Nordics and the rest of the world. Currently, the seaweed sector of Greenland is the least developed of the Nordic countries, in comparison with Canada, the Faroe Islands, Iceland or Norway. On a global scale, a comprehensive diet change towards increased seaweed consumption, and sustainable seaweed production, will have expansive effects on the SDGs. Increased seaweed mariculture alone is projected to contribute to reduced greenhouse gas emission of 0.01 to 0.02 gigatons per year by 2030 [97].

11 Conclusions and future research perspectives

11.1 Conclusions

The main objective of this PhD study was to characterise native seaweeds from Greenland regarding their potential use as a food item. The aim was therefore to determine the nutritional composition, including beneficial and anti-nutritional factors. Another objective was to investigate the influence of anthropogenic contamination on seaweed quality. Furthermore, the influence of processing in the form of washing and blanching on shelf-life of fresh seaweed was examined. Finally, this project aimed to quantify the impact of increased local seaweed harvesting and culture in Greenland within the framework of the United Nations Sustainable Development Goals (SDGs).

Overall, the ten investigated species showed a promising nutritional composition, as summarised in table 11.1, page 125, and hereafter described in more detail.

Generally, all investigated seaweed species were good sources of essential minerals and trace elements. One portion of a single-seaweed salad (of 33 g wet weight, excluding dressing) contributed with between 1 % of the recommended daily intake of Cu, Mn, P, Se and Zn and up to 55 % of the daily recommended intake of Fe and Na, with considerable differences between species.

Analysis of different thallus parts (stipe, rib, or blade) for five species (*A. clathratum*, *A. esculenta*, *L. solidungula*, *S. latissima* and *S. longicruris*) revealed that element concentrations for e.g., iodine and potassium differed greatly between different thallus parts, with no evident general pattern. This knowledge can be used to select or discard specific seaweed parts, depending on desired high or low concentrations of specific elements.

Fucus sp. samples could clearly be divided into Western (Maniitsoq, Nuuk, Qerrortusoq, Sarfannguit and Sisimiut), Southern (Narsarsuaq) and Eastern (Kangerlussuaq) origin based on principal component analysis of As, Ca, Cd, Cr, Cu, Fe, I, K, Mg, Mn, Na, Ni, P, Pb and Zn. Thus, even small sample numbers per location were sufficient to distinguish between geographical locations when investigating such an extensive range of elements simultaneously.

Fatty acid and amino acid profiles differed between species, but some patterns emerged. The fatty acid composition showed clustering into fucoids and kelp species. However, *A. clathratum* did not cluster with the other kelp species. The *Fucus* species (*F. distichus* and *F. vesiculosus*) had the highest contents of total fatty acid, n-3, and essential fatty acids, while the kelp species had the lowest concentrations of the investigated species. This is with exception of *S. latissima*, which was in the medium range. Protein content was highest in *P. palmata*, which also had the highest concentrations of amino acids ASP and GLU, which are associated with umami taste when in their free form. The dominating amino acids were ASP and GLU in all investigated seaweeds. The calculated amino acid scores were high – between 89 % for *L. solidungula* and 155 % for *S. longicruris*.

Antioxidant concentrations (TPC) and activity (DPPH, expressed as E_{50}) were highest in fucoid species (*A. nodosum*, *F. distichus* and *F. vesiculosus*), and *A. clathratum* also had a high TPC content. Therefore, these four species are the most promising candidates for the extraction of antioxidants.

Table 11.1: Nutritional composition of beneficial and anti-nutritional factors of ten seaweed species from Greenland.

Species	Nutritional composition - beneficial factors				
	Fatty acids[1]	**n-3 fatty acids**[2]	**EFA**[3]	**Proteins**[4]	**Antioxidants**
A. clathratum	→ medium	↑ high	→ medium	→ medium	↑ high
A. esculenta	↓ low	→ medium	→ medium	→ medium	→ medium
A. nodosum	↑ high	→ medium	→ medium	↓ low	↑ high
F. distichus	↑ high	↑ high	↑ high	→ medium	↑ high
F. vesiculosus	↑↑ highest	↑↑ highest	↑↑ highest	→ medium	↑↑ highest
H. nigripes	↓↓ lowest	↓↓ lowest	↓ low	↑ high	↓ low
L. solidungula	↓ low	↓ low	↓ low	↓↓ lowest	↓ low
P. palmata	↓ low	↑ high	↓↓ lowest	↑↑ highest	↓ low
S. latissima	→ medium	→ medium	→ medium	→ medium	→ medium
S. longicruris	↓ low	↓ low	↓ low	→ medium	→ medium

Species	Nutritional composition - anti-nutritional factors			
	As, Cd, Hg, Pb	**Iodine**	**Kainic acid**	**Potassium**[5]
A. clathratum	✓no concern	! high	✓no concern	✓no concern
A. esculenta	✓no concern	! high	✓no concern	✓no concern
A. nodosum	✓no concern	! high	✓no concern	✓no concern
F. distichus	✓no concern	! high	✓no concern	✓no concern
F. vesiculosus	✓no concern	! high	✓no concern	✓no concern
H. nigripes	✓no concern	! high	✓no concern	! high
L. solidungula	✓no concern	! high	✓no concern	! high
P. palmata	✓no concern	✓no concern	? present but no recommended intake levels exist	✓no concern
S. latissima	✓no concern	! high	✓no concern	✓no concern
S. longicruris	✓no concern	! high	✓no concern	! high

[1] Fatty acid median concentrations ± median absolute deviation (mg kg^{-1}dw): lowest 5 130 ± 1 370; low < 10 000; medium > 14 000; high >20 000; highest 37 500 ± 7 870.
[2] n-3 fatty acid median concentrations ± median absolute deviation (mg kg^{-1}dw): lowest 659 ± 1 214;, low < 2 000; medium > 2 000; high >3 000; highest 4 060 ± 150.
[3] Essential fatty acis (EFA, 18:3 (n-3) + 18:2 (n-6)), median concentrations ± median absolute deviation (mg kg^{-1}dw): lowest 400 ± 205; low < 1 500; medium > 1 500; high >5 000; highest 5 900 ± 939.
[4] Protein (% dw): lowest 4.6 ± 1.7; low < 5; medium > 5; high > 7; highest 12 ± 3.
[5] Potassium is an important constituent of the human diet, for patients on a low potassium diet (2 to 3 g day^{-1}), these seaweeds contribute a substantial part of the recommended daily intake, when eating a 33 g seaweed dish.

High iodine concentrations were identified as an issue for all species except for *P. palmata*, when consuming seaweed in quantities of a salad, or side dish, of 33 g as described above, because the recommended daily intake of 600 µg was exceeded by a factor of 2 for *F. vesiculosus* up to a factor of 40, for *L. solidungula*. However, recent research in East Greenland suggests that iodine bioavailability may only be around 50 %, and that excretion levels correlated with

reported seaweed consumption of the study participants, who were local Greenlanders [4]. Traditional Inuit food items have a high iodine content [4], and current research has already shown that Greenlandic Inuit possess genetic and physiological adaptations to a diet rich in polyunsaturated fatty acids [81]. Thus, adaptations regarding the iodine metabolism may also exist. Therefore, seaweeds may be an important, but not necessarily questionable, contribution to the iodine uptake of individuals who eat them. Nevertheless, it is recommendable to employ iodine-reducing preparation methods such as soaking or blanching, which have successfully been tested in other studies.

The hazardous contaminants typically associated with seaweeds (arsenic, cadmium, lead, and mercury) were below threshold values and are therefore concluded to be of no concern for seaweeds harvested in Greenland. However, for patients on a low potassium diet, *H. nigripes*, *L. solidungula* as well as *S. longicruris* may contribute significantly to the recommended daily intake (2 to 3 g/day [41]). Despite the consistent concentrations below threshold values, a clear influence of anthropogenic contamination could be seen on the elemental profiles.

Kainic acid was detected in both fresh and dried samples of *P. palmata* from Sarfannguit, Sisimiut and Nuuk. However, the detected levels were unlikely to constitute a health hazard.

Regarding the influence of local anthropogenic contamination on seaweed microbiota, we found that seaweed collected within one hundred metres of municipal outlets and sewage dumps showed contamination with human pathogens. However, there was no consistent relationship between microbiota counts on seaweed and in seawater, therefore sampling of seawater is not a suitable proxy to assess the possible contamination of the seaweed. Quantification through 16S rRNA qPCR revealed bacterial abundance of 8.9 ± 0.1 to 10.1 ± 0.0 log gene copies/g seaweed, with no evident difference between contaminated and unpolluted locations. For all samples, the main bacterial classes were *Alphaproteobacteria*, *Bacteroidia* and *Gammaproteobacteria*, and the two major bacterial families were *Flavobacteriaceae* (which includes some known fish and human pathogens) and *Rhodobacteraceae* (which includes many aquatic bacteria). Based on these results, it is recommended to establish exclusion zones around wastewater outlets, where seaweed harvesting would be banned.

Regarding the shelf-life study of washed and blanched *S. latissima*, both microbial and sensory evaluation pointed towards a maximum shelf life of 7 days. Bacterial spoilage was driven by *Pseudomonas* spp. and *Shewanella* spp., while actinomycetes, yeast and moulds were less prominent. Blanching successfully increased odour attributes such as “boiled peas”, “pleasantly sour” and “umami”, and changed the colour from a dark greenish brown to a dark green, opening interesting possibilities for culinary experiments and product development.

The semi-quantitative analysis of the impacts of increased seaweed harvesting and cultivation in Greenland revealed that SDG 13 (Climate action) would be most negatively impacted by the increased consumption of fossil fuels used for harvesting, processing, and exporting of seaweeds. Most positively impacted were SDG 8 (Decent work and economic growth), SDG 12 (Responsible consumption) and SDG 14 (Life below water). It is therefore recommended to mitigate the negative impact on SDG 13 by reducing the energy consumption through more efficient vessels, good vessel maintenance and improved processing methods, as well as a shift

to renewable energy sources. Finally, it is recommended to support the decision and management makers through providing them with the knowledge generated in this project. Thereby, the public health program Inuuneritta III can incorporate seaweed, fostering nationwide uptake and increase in local seaweed consumption. Furthermore, seaweed can advantageously be put onto the SDG agenda of Greenland, which is currently under development.

The following passages are summarizing accessibility, cultivation potential, and regulatory requirements. These aspects were not specifically studied in the present work, but are crucial to consider.

Species growing in the littoral zone (*A. nodosum*, *F. distichus*, *F. vesiculosus* and *P. palmata*) can be harvested quite easily from the shore at low tide, thus requiring no specialised equipment. In contrast, the species growing in the sublittoral zone (*A. clathratum*, *A. esculenta*, *H. nigripes*, *L. solidungula*, *S. latissima* and *S. longicruris*) require equipment for harvesting. While the latter equipment can be as simple as a boat and a tool to cut off, rake or otherwise detach the seaweed, it still requires some investment.

The cultivation of both *A. esculenta* and *S. latissima* has been successful in experimental scale in Greenland by both researchers from the Greenland Institute of Natural Resources and the company Royal Greenland A/S. Cultivation methods for other species, such as *F. vesiculosus* and *P. palmata*, are currently being developed in other European and Nordic countries. Therefore, the future cultivation of additional species in Greenland is definitely possible, building upon the knowledge gathered elsewhere.

Regarding the EU Novel food regulation, *A. clathratum*, *F. distichus*, *H. nigripes* and *L. solidungula* fall under the category of novel foods and would therefore require certification to comply with the food regulation for the EU market. While this makes these species less interesting for international export, *A. clathratum* and *F. distichus* have interesting nutritional compositions, including antioxidant levels, which may make certification worthwhile.

In conclusion, the current study provides a solid foundation for the development of a knowledge base on seaweeds from Greenland as foods. While there is not one species that is the perfect solution for every application, the present study provides a good starting point to select a species, be it for household collection or the commercial extraction of antioxidants. When harvesting and culturing, areas close to, and downstream of, anthropogenic contamination sources should be avoided. Hopefully, the present work will stimulate the consumption and production, both through harvesting and cultivation, of seaweed in Greenland and beyond.

11.2 Future research perspectives

There are many truly relevant issues to be investigated further, which can be loosely grouped into consumer studies, nutritional composition, post-harvest processing and cultivation aspects.

11.2.1 Consumer studies

This project included a very limited scoping of Greenlandic consumer interests and opinions. To understand the current consumption patterns and consult local, indigenous knowledge on seaweed, further studies are needed. They could also be organised as citizen science projects, activating the storytelling traditions, and connecting old and young. It is also highly recommended to include young Greenlanders into such studies, as they are very interested in the development of local Greenlandic products and solutions, as expressed in a workshop on biodiversity in Nuuk in September 2020 [150].

11.2.2 Nutritional composition

There are many aspects of the nutritional composition of Greenland seaweeds not covered in this PhD project, which should be investigated further. Just to name a few examples: pigments, polysaccharides (e.g., alginate, carrageenan or fucoidan) and vitamins, etc.

Bioavailability studies, especially for iodine but also for amino acids, to determine the true ileal digestibility and therefore their quality as a protein source, should also be carried out.

Since we found some indications for regional differences, it is recommended to further investigate the geographical influence on seaweed genetics, and nutritional composition, especially considering the vast area of Greenland.

As numerous studies from other regions have demonstrated, the constituents of seaweeds may be highly variable, depending on the season. Therefore, seasonal differences should also be investigated. This is especially important with regard to cultivation, where a specific component may be decisive for the timing of harvest.

Finally, there are many more species to be found in Greenland, some of which could be interesting for food, functional food, or medicinal uses. Here it would be appropriate to start out with a survey of traditional uses for seaweed in Greenland and possibly neighbouring regions, e.g., Nunavut, Canada, to identify additional suitable species.

11.2.3 Post-harvest processing

Firstly, it is recommended to repeat the shelf-life study of washed and blanched sugar kelp *S. latissima* in Greenland, to verify the results in a different region of the planet, grown in a vastly different climate and salinity regime.

Drying methods are also a highly relevant and interesting aspect to study. Firstly, because drying is one of the most common preservation methods traditionally used in Greenland. Secondly, because seaweed is often brought to the market as a dried product and is therefore a format that consumers are used to. Thirdly, because seaweed lends itself well to drying and rehydration. Last but certainly not least, because export of seaweed from Greenland would greatly benefit from the reduced weight and volume of dried seaweed, versus for example, frozen seaweed. Here it could be meaningful to combine the experiments with the use of renewable energy sources such as hydropower and wind power, to combat the negative impact

on SDG 13 (Climate action) from the energy required for drying. In addition, the effect of drying on the nutritional composition needs to be quantified.

Fermentation of seaweed is another interesting process to investigate. Some studies on Danish seaweed are currently in progress (e.g., Sea Bioassess) and a Danish company (Nordisk tang) has already intermittently brought a fermented seaweed salad onto the market.

For the application of seaweed extracts as preservatives in food systems, ethanolic/supercritical water extracts would be interesting to investigate.

11.2.4 Harvesting aspects

Guidelines for harvesting should be developed, which include best practices to obtain optimal quality and yields and reduce the negative impacts on the ecosystem.

Further research is also recommended to create guidelines for the distance between anthropogenic contamination sources such as sewage outlets and location of seaweed harvest.

11.2.5 Cultivation aspects

Experimental cultivation is already taking place for *A. esculenta* and *S. latissima*, but upscaling will require more knowledge that could be gained from targeted experimentation. Cultivation techniques for other species may be partly transferable from other (Nordic) countries but need more research to adapt them for successful use in Greenland. Crucially, the focus must be on locally sourced genetic material to benefit from local adaptations as well as to keep endemic populations and biodiversity unaffected.

Just as for harvesting, guidelines for the distance between anthropogenic contamination sources the location of cultivation sites should be developed based on further research.

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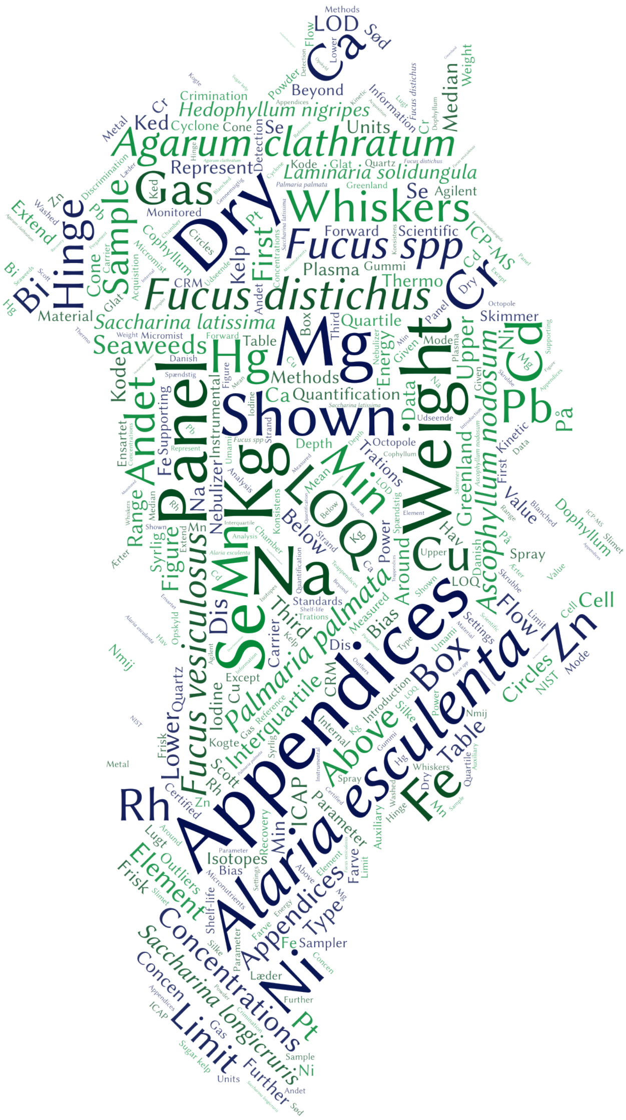
Appendices


Appendices
Alaria esculenta
Weight
Panel
Kg
Shown
Mg
Dry
Na
Zn
Se
Ni
Fucus distichus
Fucus spp
Agarum clathratum
Whiskers
Hinge
Sample
Gas
Cr
Cd
Pb
Hg
Cu
Fe
Rh
Bi
Ca
LOQ
Min
Mn
Box
Andet
Fucus vesiculosus
Palmaria palmata
Ascophyllum nodosum
Saccharina latissima
Saccharina longicruris
Laminaria solidungula
Hedophyllum nigripes
Concentrations
Limit
Element
Above
Interquartile
Seaweeds
Circles
Flow
Greenland
Upper
Lower
First
Third
Methods
Quantification

A Supporting information - Micronutrients and iodine

Table A.1: Instrumental settings for the ICP-MS methods.

Parameter	Type/Value			
	Agilent 8900 ICP-QQQ-MS			Thermo Scientific iCAP Q ICP-MS
Sample introduction				
Nebulizer	MicroMist			PFA- ST
Spray chamber	Scott-type			Cyclone Quartz
Skimmer and sampler cone	Pt			Ni
Plasma parameters				
Forward power	1550 W			1550 W
Plasma gas flow	15.0 L min^{-1}			15 $Lmin^{-1}$
Carrier gas flow	1.03 L min^{-1}			
Nebulizer gas	0.99 L min^{-1}			1.0 $Lmin^{-1}$
Auxiliary gas	0.9 L min^{-1}			0.8 $Lmin^{-1}$
Sample depth	8.0 mm			2.5 mm
Cell parameters				
Gas flow	4.8 mL He min^{-1} (Ca, Mg, Na, K), 5.0 mL He min^{-1} (Cd, Cr, Cu, Fe, Hg, Ni, Zn)	30 % O_2	No gas	5.1 mL He min^{-1}
Octopole bias	-18.0 V	-3.0 V		
Energy discrimination	5.0 V	-7.0 V		Kinetic Energy Discrimination (KED) mode
Data acquisition parameters				
Isotopes monitored	^{44}Ca, ^{114}Cd, ^{52}Cr, ^{65}Cu, ^{56}Fe, ^{202}Hg, ^{39}K, ^{24}Mg, ^{55}Mn, ^{23}Na, ^{60}Ni, ^{66}Zn	$^{75}As \rightarrow {}^{75}As^{16}O$, $^{31}P \rightarrow {}^{31}P^{16}O$, $^{80}Se \rightarrow {}^{80}Se^{16}O$	^{208}Pb	^{127}I
Isotopes of internal standards	^{209}Bi, ^{115}In, ^{103}Rh	$^{103}Rh \rightarrow {}^{103}Rh$	^{209}Bi, ^{115}In, ^{103}Rh	^{125}Te

Table A.2: Limit of detection (LOD), limit of quantification (LOQ), and reference material analysis. All units are mean values, in mg kg^{-1}, except for Ca, K, Mg, Na and P which are in g kg^{-1}, Recovery (R) is given in %.

			NMIJ CRM 7405-a			NIST 3232 Kelp powder		
Element	LOD	LOQ	certified	measured	R	certified	measured	R
As	0.11	0.35	35.8 ± 0.9	36.53 ± 1.24	102	38.3 ± 1.3	38.16 ± 0.72	100
Ca	0.37	1.23	15.2 ± 0.3	22.36 ± 1.47	147	12.26 ± 0.68	16.89 ± 0.71	138
Cd	0.0007	0.0022	0.79 ± 0.02	0.82 ± 0.03	104	0.43 ± 0.01	0.43 ± 0.0046	100
Cr	0.06	0.22	3.4 ± 0.1	3.4 ± 0.19	100	5.92 ± 0.52	5.14 ± 0.24	87
Cu	0.55	1.84	1.55 ± 0.07	1.77 ± 0.19	114	3.88 ± 0.09	3.56 ± 0.18	92
Fe	9.7	32	311 ± 11	319 ± 13.98	102	672 ± 13	672 ± 0.81	100
Hg	0.02	0.08	NA	0.05 ± 0.0042	NA	0.11 ± 0.0032	0.09 ± 0.0041	81
I	50	167	NA	NA	NA	944 ± 88	822 ± 9	87
K	0.59	1.98	47.5 ± 0.7	48.6 ± 2.24	102	76 ± 1.1	74.58 ± 1.79	98
Mg	0.06	0.2	6.79 ± 0.1	6.88 ± 0.3	101	6.13 ± 0.18	6.25 ± 0.07	102
Mn	0.06	0.21	14.1 ± 0.7	13.92 ± 0.8	99	24.6 ± 1.6	21.39 ± 0.91	87
Na	0.38	1.25	16.2 ± 0.2	15.73 ± 0.97	97	16.33 ± 0.38	14.16 ± 0.23	87
Ni	0.1	0.33	2.2 ± 0.1	2.2 ± 0.07	100	NA	2.6 ± 0.08	NA
P	0.02	0.06	1.01 ± 0.03	0.78 ± 0.06	77	4.55 ± 0.05	± 3.92 0.27	86
Pb	0.02	0.07	0.43 ± 0.03	0.44 ± 0.02	102	1.03 ± 0.04	0.99 ± 0.03	96
Se	0.03	0.11	NA	0.08 ± 0.02	NA	NA	0.04 ± 0.01	NA
Zn	0.66	2.19	13.4 ± 0.5	14.11 ± 0.76	105	27.4 ± 1.1	26.74 ± 0.73	98

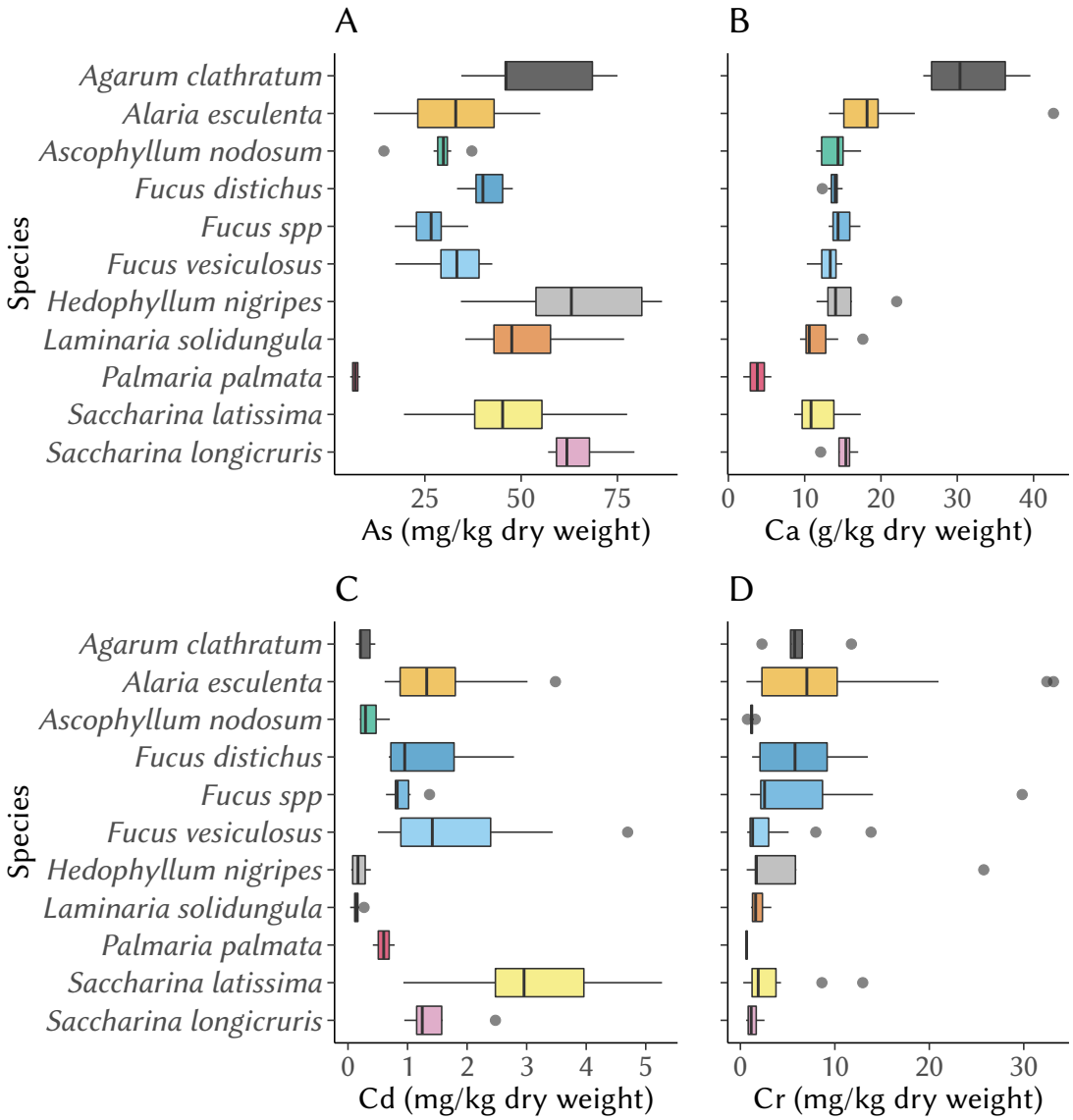


Figure A.1: Concentrations of elements (Panel A: As, panel B: Ca, panel C: Cd, panel D:Cr)) in Greenland seaweeds: *Agarum clathratum*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus distichus*, *Fucus* spp,*Fucus vesiculosus*, *Hedophyllum nigripes*, *Laminaria solidungula*, *Palmaria palmata* and *Saccharina latissima*. Concentrations of Hg were below the limit of quantification (LOQ) of 0.078 mg/kg dry weight in all samples, and are not shown. Concentrations of Se are only shown for samples above the LOQ of 0.111 mg/kg dry weight. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range. Outliers beyond the whiskers are shown as circles.

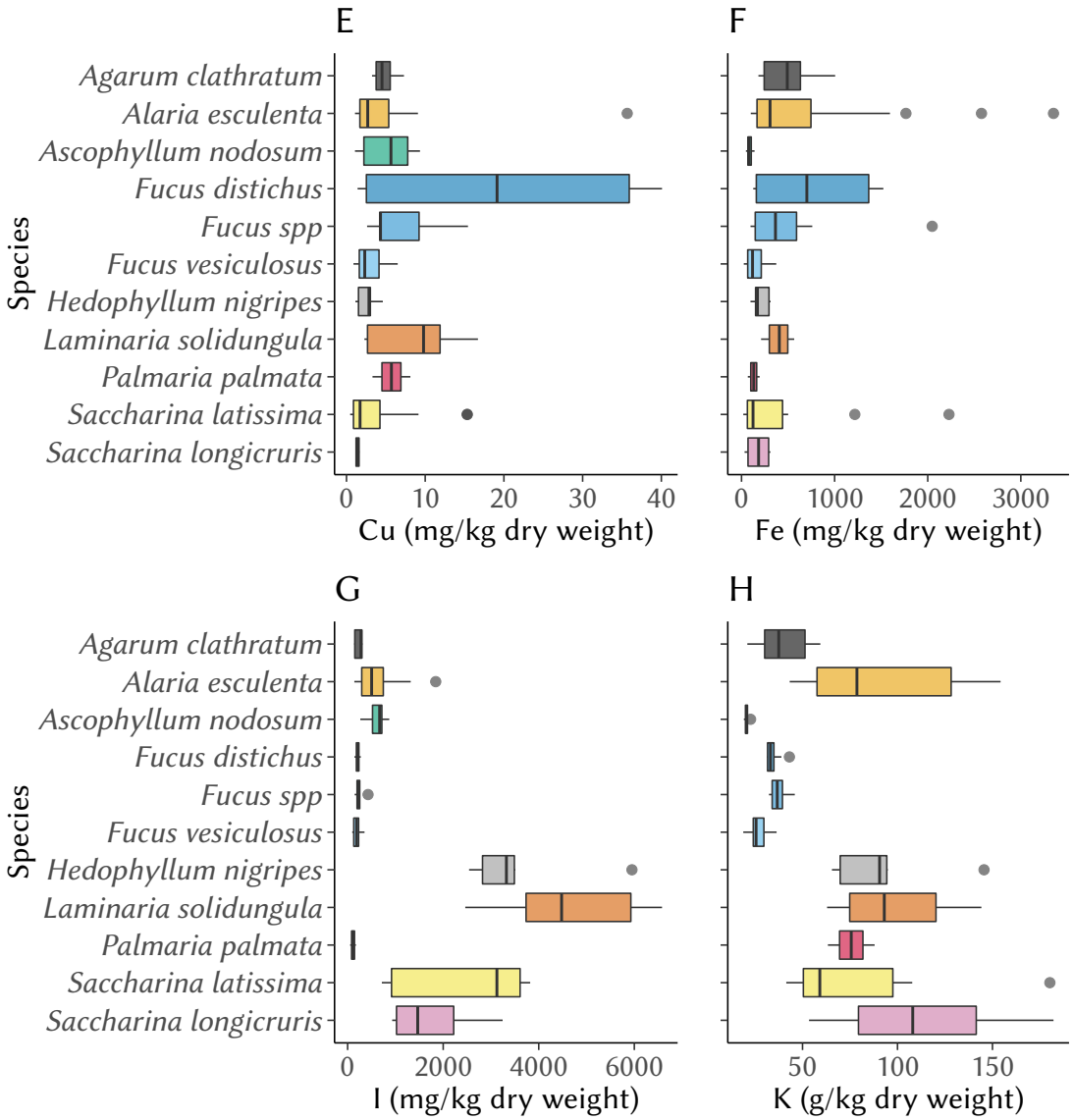


Figure A.2: Concentrations of elements (Panel E: Cu, panel F: Fe, panel G: I, panel H: K)) in Greenland seaweeds: *Agarum clathratum*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus distichus*, *Fucus* spp,*Fucus vesiculosus*, *Hedophyllum nigripes*, *Laminaria solidungula*, *Palmaria palmata* and *Saccharina latissima*. Concentrations of Hg were below the limit of quantification (LOQ) of 0.078 mg/kg dry weight in all samples, and are not shown. Concentrations of Se are only shown for samples above the LOQ of 0.111 mg/kg dry weight. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range. Outliers beyond the whiskers are shown as circles.

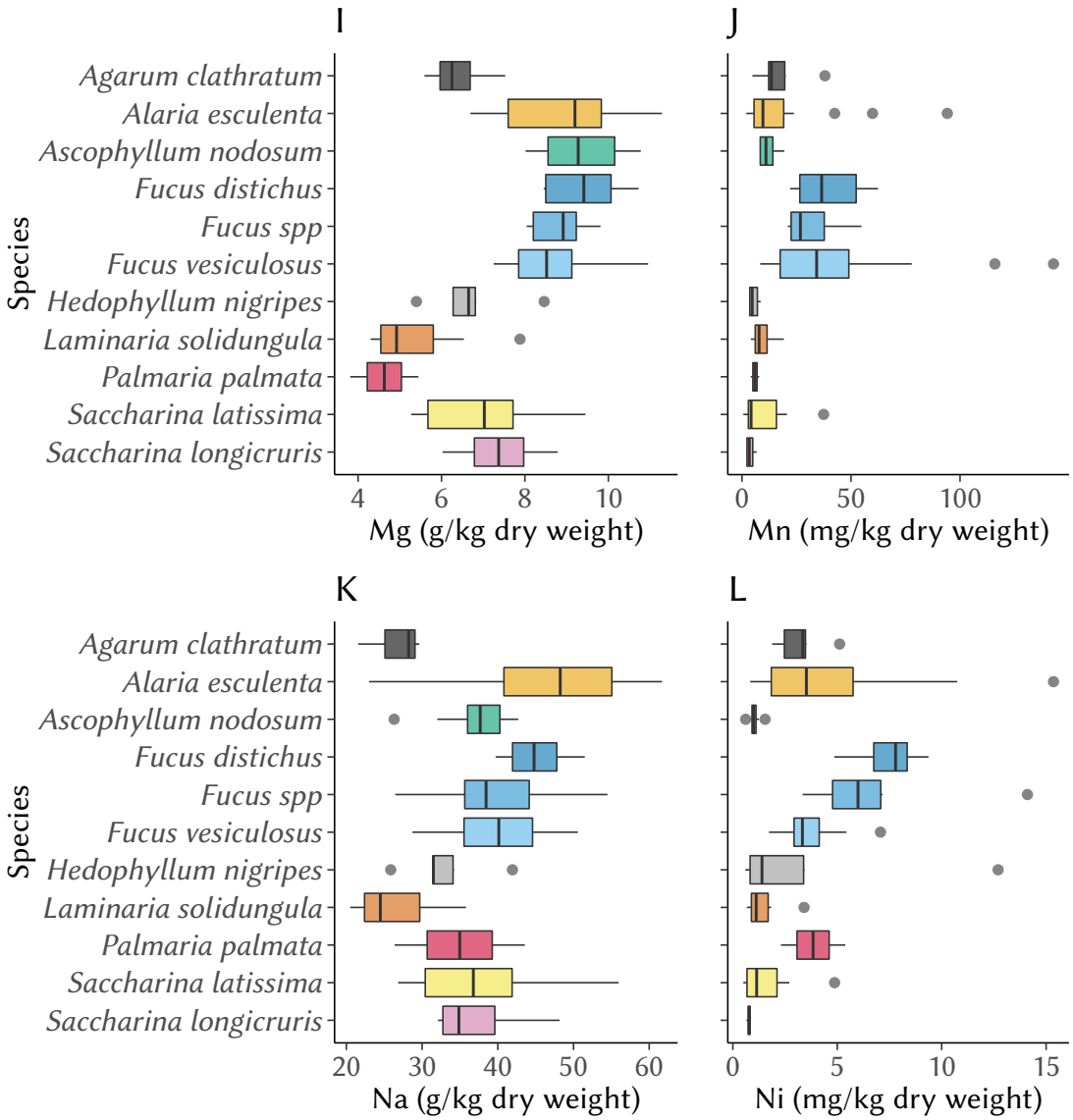


Figure A.3: Concentrations of elements (Panel I: Mg, panel J: Mn, panel K: Na, panel L: Ni)) in Greenland seaweeds: *Agarum clathratum*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus distichus*, *Fucus* spp,*Fucus vesiculosus*, *Hedophyllum nigripes*, *Laminaria solidungula*, *Palmaria palmata* and *Saccharina latissima*. Concentrations of Hg were below the limit of quantification (LOQ) of 0.078 mg/kg dry weight in all samples, and are not shown. Concentrations of Se are only shown for samples above the LOQ of 0.111 mg/kg dry weight. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range. Outliers beyond the whiskers are shown as circles.

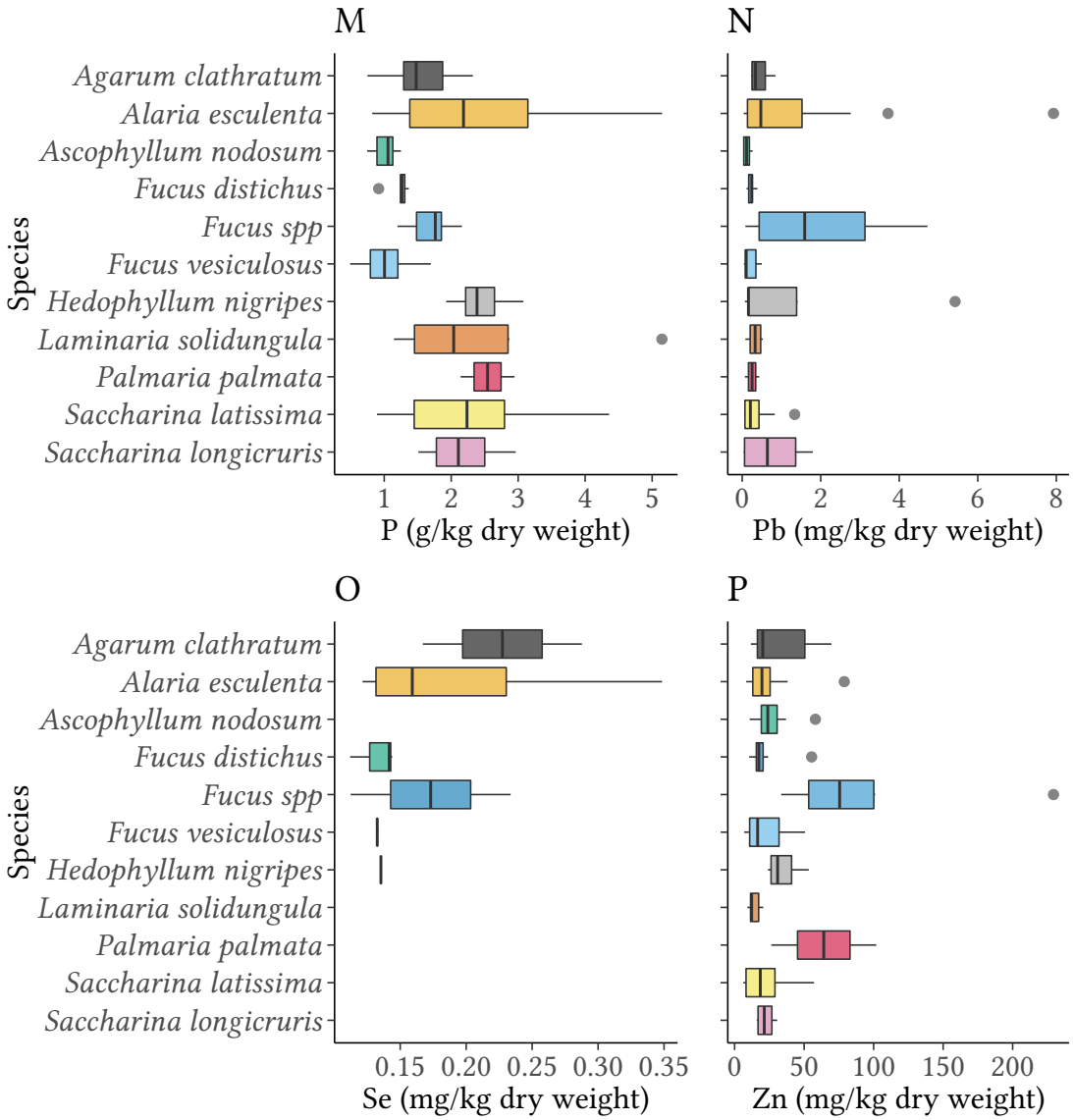
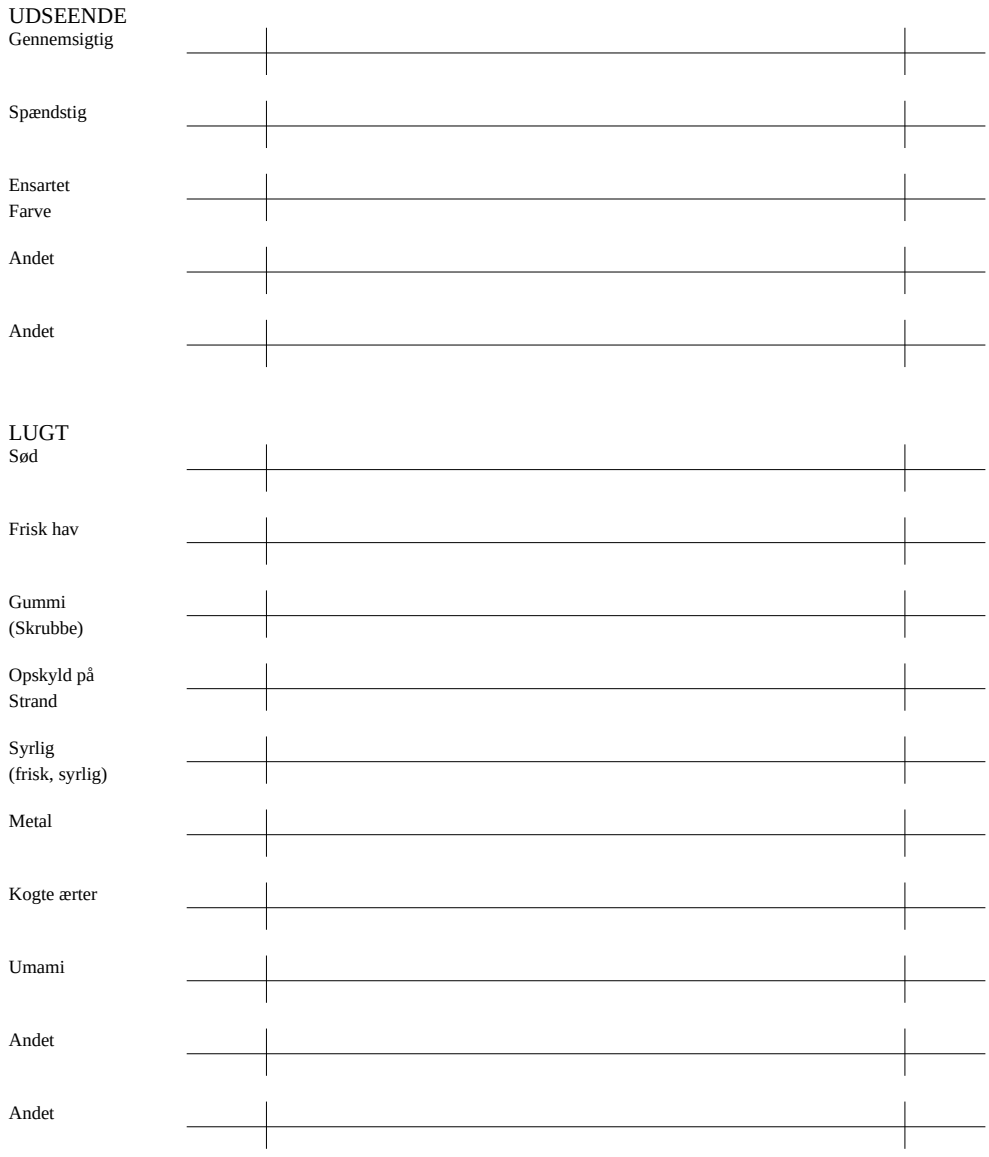


Figure A.4: Concentrations of elements (Panel M: P, panel N: Pb, panel O: Se, panel P: Zn)) in Greenland seaweeds: *Agarum clathratum*, *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus distichus*, *Fucus* spp,*Fucus vesiculosus*, *Hedophyllum nigripes*, *Laminaria solidungula*, *Palmaria palmata* and *Saccharina latissima*. Concentrations of Hg were below the limit of quantification (LOQ) of 0.078 mg/kg dry weight in all samples, and are not shown. Concentrations of Se are only shown for samples above the LOQ of 0.111 mg/kg dry weight. The lower and upper hinges of the box represent the first and third quartile, around the median; the whiskers extend no further from the hinge than 1.5 * inter-quartile range. Outliers beyond the whiskers are shown as circles.

B Supporting information - Shelf-life of washed or blanched Danish sugar kelp

Kode:



Kode:

KONSISTENS