Codium fragile subsp. *fragile* and *Sargassum muticum*: Non-native seaweeds in a changing environment

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Acknowledgements

My work on this topic began when I started as a master's student at the University of Bergen. I had just arrived in Norway, and had little idea of what I wanted to focus on – but knew that I was interested in impacts of anthropogenic change. When I saw that there was someone in the Marine Biodiversity group with 'invasive species' listed as a research interest, I decided to arrange a visit there, and ended up staying for 7 years.

Seaweeds were not a great interest of mine when I began – there was not much focus on them in my bachelor course, and I had never lived by the sea before arriving in Bergen. But as I began working on non-native seaweeds and learning about the different seaweeds in Norway, I started to see what a fascinating group they are. In addition, far from seaweed being the smelly, slimy stuff I remember washed up on beaches during my childhood, I discovered that many species are remarkably beautiful.

This 'conversion' was in large part due to the influence of Kjersti Sjøtun, my supervisor, whose enthusiasm for seaweeds is contagious. However, being interested in what you study is only part of the battle when it comes to completing a thesis. I was fortunate in that Kjersti is also a consistently good supervisor, who guided my work to make it productive and enjoyable. She has been gently critical when necessary, and engaged and encouraging with my work the whole way. I want to thank her for this, and for making me feel like we were always working as a team – and not least for putting up with a 'landkrabbe' like me on our fieldwork!

However, she is just one of the many people who have helped me. Without them much of my work would have been impossible, or at least taken twice as long! This includes my co-supervisor, Vivian Husa, who was good company on trips and always had a realistic but encouraging attitude to new ideas. In particular I appreciate her support for the 'extra' things I wanted to do regarding *Sargassum* and nutrients, and *Codium* taxonomy. There are also several staff members from the University of Bergen whose assistance has been indispensable. I would like to thank Louise Lindblom for her guidance in the DNA lab, Solveig Thorkildsen for helping me with DNA work and data-collection, Heikki Savolainen for assistance and advice on work in the climate rooms, Elżbieta Petelenz-Kurdziel for running last-minute CN analyses, and Tomas Sørlie for practical assistance

Abstract

Non-native species have received increasing scientific and public attention over the last three decades, and have been identified as a threat to biodiversity. However, coastal environments may also be affected by several other anthropogenic stressors, including climate change, eutrophication, overfishing and coastal development. How such stressors interact is difficult to predict and, especially in the marine environment, understudied. In particular, it is uncertain how climate change may facilitate non-native seaweeds. This is important as seaweeds have a key role in coastal temperate ecosystems.

The aim of this thesis was therefore to examine factors affecting the success of two common and widespread non-native seaweeds, and whether environmental change will benefit them. The green seaweed *Codium fragile* subsp. *fragile* (Suringar) Hariot and brown seaweed *Sargassum muticum* (Yendo) Fensholt were chosen as both are canopy-forming species and can be abundant in the infra- and sublittoral in southwestern Norway. They both have their northern limits along the Norwegian coast, which along with their relatively high temperature optima suggests that warming may facilitate them in Norway. However, their success may also be limited by abiotic or biotic conditions which could continue to limit them despite environmental changes occurring.

For *C. fragile* subsp. *fragile*, I investigated how abundance and/or local distribution was related to wave-exposure (within a semi-exposed to sheltered range), hard substratum type, disturbance of existing canopy seaweeds, and a native fucoid (*Fucus serratus* Linnaeus). In terms of environmental change, I examined how increases in minimum and maximum temperatures would affect the growth and abundance of the alga. These factors were assessed by field surveys, with the exception of disturbance and temperature where effects were also/instead assessed experimentally. For *S. muticum*, I experimentally investigated how its cover was influenced by disturbance of existing canopy seaweeds, and how growth and survival of laterals was affected by competition in canopies with two native seaweeds (*F. serratus* and the kelp

Saccharina latissima C.E. Lane, C. Mayes, Druehl & G.W. Saunders). For environmental changes, I studied whether nutrient enrichment or increased summer temperatures could affect growth, survival or competitive relationships.

Non-native and native subspecies of *C. fragile* have frequently been misidentified, thus to ensure that my studies were carried out on the non-native subspecies I genetically sequenced populations along the coast and in herbaria. This revealed that subsp. *atlanticum* is present in Norway, but most *C. fragile* in southwestern Norway is the non-native subspecies, subsp. *fragile*. The morphological traits investigated could be variable and overlap between the subsp. *atlanticum* and subsp. *fragile*, indicating that these are not reliable for identification here.

With regard to abiotic and biotic factors affecting success, *C. fragile* subsp. *fragile* (hereafter *C. fragile*) was most successful at stony sites (cobble/boulder substratum). This substratum type was associated with higher abundances, higher persistence over time, and deeper lower depth limits than on bedrock, where *C. fragile* populations tended to be transient and limited to the infralittoral. Exposure also played a role; it did not affect abundance, but *C. fragile* distribution tended to expand closer to the surface at more sheltered sites. At stony sites *C. fragile* could persist as the dominant canopy for at least 5 years.

The vertical distributions of *C. fragile* and *Sargassum muticum* are mostly infra- and sublittoral, overlapping with those of *F. serratus* and some native kelp species (commonly *Saccharina latissima* and *Laminaria digitata* (Hudson) J.V. Lamouroux). Distribution patterns suggested that *C. fragile* may limit *F. serratus*, but only in the infralittoral zone at favourable sites. Disturbance of canopy seaweeds did not lead to an increase in *C. fragile* or *Sargassum muticum* cover, but this may be due to the short length of the disturbance, the heterogeneous nature of the infralittoral fringe habitat, and propagule pressure in the case of *Sargassum muticum*. In mature mixed canopies, *Saccharina latissima* and *Fucus serratus* did not limit the growth of *Sargassum muticum* laterals, but these native species did have a negative effect on survival of the laterals.

Observations suggest that some local factor(s) may limit the growth and condition of *Sargassum muticum* in summer, and this likely affected the competitive relationships documented here. Data were consistent with cover of filamentous brown epiphytes (Ectocarpales) having a negative impact, but also suggested that *S. muticum* is nitrogen limited. Nutrient enrichment was associated with improved condition of *S. muticum* thalli at one site, but not at another where filamentous epiphytes were abundant. More work is needed on this topic.

Higher temperatures in winter/spring are likely to favour *C. fragile* by increasing the growth rate and persistence of populations, although a longer study is needed to confirm the latter. However, a direct impact of higher summer temperatures was not detected for *C. fragile*, and it is likely that the rapid shortening of days in October may prevent any temperature-driven increases in growth rate in autumn. *Sargassum muticum* also showed little direct benefit of increased summer temperature on growth or condition, suggesting that hotter summers will not improve condition where local limiting factors are present. However, higher summer temperatures affected competitive relationships between canopy species in the upper sublittoral: *Saccharina latissima* was negatively affected by the heat, reducing its competitive effects on *Sargassum muticum* and the native *F. serratus*. Under these conditions, *F. serratus* grew and survived well.

The work of this thesis provides basic distribution and growth rate information about these non-native species in Norway, and documents their associations with abiotic and biotic factors, including their competitive relationships with two common native seaweeds. It shows that the response of warm-adapted non-native species to temperature increases can vary by season and local conditions.

List of publications

Paper I

Armitage CS, Sjøtun K (2016) *Codium fragile* in Norway: subspecies identity and morphology. Botanica Marina 59:439-450, doi: 10.1515/bot-2016-0095

Paper II

Armitage CS, Sjøtun K, Jensen KH (2014) Correlative evidence for competition between *Fucus serratus* and the introduced chlorophyte *Codium fragile* subsp. *fragile* on the southwest coast of Norway. Botanica Marina 57:85-97, doi: 10.1515/bot-2013-0087

Paper III

Armitage CS, Sjøtun K (2017) Can an old alien benefit from rising ocean temperatures? An experimental and field study on the growth and local distribution of *Codium fragile* subsp. *fragile* (Chlorophyta). Marine Biology 164:142, doi: 10.1007/s00227-017-3170-5

Paper IV

Armitage CS, Sjøtun K. Impacts of canopy disturbance on non-native seaweed abundance. (*Unpublished manuscript*)

Paper V

Armitage CS, Husa V, Petelenz-Kurdziel EA, Sjøtun K (2017) Growth and competition in a warmer ocean: a field experiment with a non-native and two native habitat-building seaweeds. Marine Ecology Progress Series 573:85-99, doi: 10.3354/meps12161

All photos and diagrams in this thesis are taken/created by the author, unless otherwise specified.

1.   Introduction

1.1 Seaweeds in a changing environment

The earth is currently undergoing an unprecedented period of rapid change, with humans having such large and widespread impacts that the "Anthropocene" has been proposed as a new geological epoch (Lewis & Maslin 2015). Several types of environmental change can disrupt communities and ecosystems: for seaweeds and coastal systems, these include nutrient enrichment, hypoxia, sedimentation, sea-level rises, extreme weather, ocean acidification, temperature changes, over-grazing, and coastal development (Brodie et al. 2014, Mineur et al. 2014, Wong et al. 2014) (Fig. 1). Of these, both climate-related changes and species introductions have global impacts on biodiversity. Climate change may result in changes in species ranges, leading to the formation of novel communities (Garcia et al. 2014), while non-native species affect communities through modification of habitats, and contribute to 'homogenisation' of the global biosphere (Simberloff et al. 2013). Such impacts on biodiversity can affect ecosystem functioning, and ecosystem services which humans rely on (Cardinale et al. 2012).
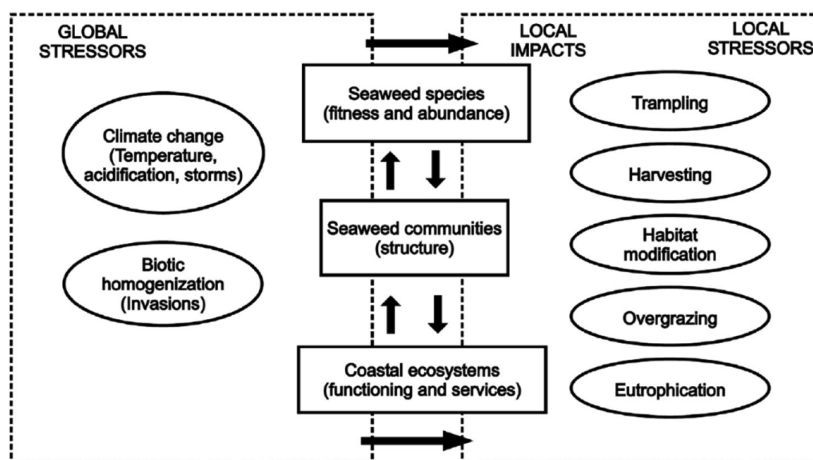
Figure 1 A conceptual framework of stressors faced by European seaweeds. This divides stressors into 'global' and 'local', with local stressors being those that can be controlled most readily by local management actions. Both global and local stressors can affect other local stressors, as well as affecting the seaweeds. *Reprinted from "European seaweeds under pressure: Consequences for communities and ecosystem functioning," by Mineur et al. (2014), Journal of Sea Research, volume 98, p. 93. Copyright 2014, with permission from Elsevier.*

Organisms have been introduced to new habitats by humans intentionally and unintentionally for centuries, and some non-native species are considered beneficial for humans, e.g. aquaculture species. However, the negative effects of non-native species have come increasingly into focus in recent decades, and invasion biology has grown as a field (Simberloff et al. 2013). Unfortunately, despite a large increase in the study of biological introductions since the early 1990s, marine non-native species remain relatively understudied (Lowry et al. 2013). This is also the case for climate change research, where documentation of marine effects is relatively poor, despite indications that impacts are strong in the oceans (Richardson & Poloczanska 2008, Poloczanska et al. 2013). Marine and terrestrial systems are not identical ecologically, and oceans face unique threats (e.g. acidification), thus it is essential that changes in marine environments receive more attention (Richardson & Poloczanska 2008).

Within temperate marine coastal environments, seaweeds (macroalgae) are ecologically vital, with key roles in ecosystem functioning. One of these roles is that they provide habitat, with seaweed beds able to support very diverse and abundant communities of associated taxa (Fredriksen et al. 2005, Christie et al. 2009). Many species depend on the physical structure of seaweeds (Christie et al. 2009), or on their modification of abiotic conditions (e.g. Thomsen et al. 2010, Pocklington et al. 2017). Seaweeds also play a critical role as primary producers at the base of food webs. Around 30 % of the carbon they fix is transferred to herbivores, and another 10-80 % is transported to other habitats (Duarte & Cebrián 1996), where seaweed detritus supports secondary production (Krumhansl & Scheibling 2012a).

Furthermore, seaweeds are economically valuable. In the NE Atlantic they are harvested for fertiliser and food, used as cosmetic, food, textile and pharmaceutical ingredients, and have potential as biofuels (Beaumont et al. 2008, Smale et al. 2013). Norway alone harvests up to 180000 tonnes of the kelp *Laminaria hyperborea* annually (Vea & Ask 2011). They also provide ecosystem services; for example, reduction of wave-damage, creation of habitat for commercial fishery species (e.g. lobster, *Homarus gammarus*, and juvenile Atlantic cod, *Gadus morhua*), and creation

of habitats which support nature-based tourism such as diving and fishing (Beaumont et al. 2008, Smale et al. 2013, Gundersen et al. 2016).

Because of these economic and ecological roles, the impact of environmental changes on seaweeds could have far-reaching effects (Harley et al. 2012). Environmental changes (Fig. 1) can affect seaweeds in several ways: they can have direct effects on seaweed survival, growth and reproduction, or indirect effects via alterations to competition, herbivory, epiphytism and pathogens (Harley et al. 2012). They can also affect the ability of seaweeds to provide food and habitat (e.g. Krumhansl et al. 2014, Simonson et al. 2015a). The resulting changes in North Atlantic seaweed communities are expected to be significant, with decreases in calcified algae, decreases in or changes in the composition of perennial canopy seaweeds, and increases in seagrasses, annual kelps, non-native seaweeds, and simple turf and filamentous communities (Brodie et al. 2014)

Ocean warming in particular is expected to affect the abundances and ranges of many seaweeds (Straub et al. 2016), as temperature is a critical factor in determining seaweed distribution (van den Hoek 1982). There are several documented examples of this already occurring: higher mean temperatures are associated with changes in seaweed community composition in Australia and Japan (Wernberg et al. 2011, Tanaka et al. 2012), with changes in the abundances of large brown seaweeds in the British Isles (Yesson et al. 2015), with range shifts on the Iberian Peninsula (Lima et al. 2007, Duarte et al. 2013) and with loss of kelps in Canada (Filbee-Dexter et al. 2016). However, changes in mean temperatures are not the only aspect of climate change; there may also be changes in seasonality, or the frequency or severity of extreme events (Garcia et al. 2014). Thus while predictions of mean temperatures can be used to predict changes in seaweed distributions (e.g. Müller et al. 2009), these may underestimate changes if other aspects of climate change are not taken into account. In particular, it appears that extreme events (e.g. marine heatwaves) may have large impacts and accelerate effects of warming faster than expected from mean changes alone (Jentsch et al. 2007, Mills et al. 2013, Smale & Wernberg 2013, Wernberg et al. 2013, Wernberg et al. 2016).

In addition to their individual effects, stressors may occur simultaneously and have additive, synergistic, or antagonistic effects on seaweeds (Strain et al. 2014). For example, nutrient enrichment alone can negatively affect perennial canopy algae (Mineur et al. 2014), but can also interact with increased temperature to have synergistic negative effects (Strain et al. 2014). Interactions may also occur when one change facilitates another change. For example, overfishing of predators may remove restrictions on climate-driven range-expansions of herbivores, which then decimate kelp beds and create barrens (Ling et al. 2009). However, ocean acidification may weaken herbivores such as urchins, potentially increasing predation and aiding barren recovery (Asnaghi et al. 2013). Such interactions make predicting the impacts of environmental change challenging at the current level of knowledge.

Due to the important role of seaweeds, assessment and study of anthropogenic effects on them should be a priority. Despite this, there are large gaps in the knowledge of seaweeds, even in the well-studied NE Atlantic region. These include a lack of data on species distributions and community diversity from which changes can be assessed, and a lack of studies on the interactive impacts of multiple stressors, such as interactions between climate change and non-native seaweeds (Harley et al. 2012, Mineur et al. 2014, Davidson et al. 2015). The latter is particularly important as climate change may facilitate the expansion of non-native species which previously have been restricted, leading to larger, more widespread, or unforeseen effects (Occhipinti-Ambrogi 2007, Simberloff et al. 2013).

1.2 Non-native seaweeds and their effects

Non-native seaweeds may be defined as seaweeds which have been transported to a habitat disconnected from their native range, which they would probably not have reached without human intervention (Boudouresque & Verlaque 2002). Many different terms are used to describe these species (e.g. 'invasive', 'introduced', 'alien', 'non-native') with various definitions, which may cause confusion (Colautti & MacIsaac 2004). For neutrality I use the term 'non-native', which may be applied to any species not native to an area regardless of its impacts or abundance.

The addition of a non-native seaweed (NNS) to a habitat can have ecological or economic effects, as indicated by reviews and meta-analyses of NNS impacts (e.g. Schaffelke & Hewitt 2007, Williams & Smith 2007, Thomsen et al. 2009, Maggi et al. 2014, Thomsen et al. 2014, Davidson et al. 2015, Petrocelli & Cecere 2016, Thomsen et al. 2016). NNS can affect native communities in several ways: they can hybridise (e.g. Johnson et al. 2012) or compete with native seaweeds, and affect fauna through modification of habitats or food-webs (Schaffelke & Hewitt 2007, Davidson et al. 2015, Thomsen et al. 2016). Effects on native seaweeds are often negative, while effects on fauna vary (Thomsen et al. 2009, Maggi et al. 2014, Thomsen et al. 2014). As a group, NNS have little overall effect on the richness or diversity of fauna (Thomsen et al. 2009), but there are many examples of individual NNS affecting the composition of seaweed-associated communities via changes to the densities of member-species (Schaffelke & Hewitt 2007, Davidson et al. 2015).

It can however be difficult to generalise about the ecological effects of NNS for several reasons. Relatively few species have been studied for impacts (Davidson et al. 2015), but impacts may vary between species and recipient habitats (e.g. Buschbaum et al. 2006). There may also be effects which are challenging to record (Johnson 2007, Thomsen et al. 2016), for example, effects on ecosystem functions such as detritus exports (e.g. Krumhansl & Scheibling 2012b), or long-term effects on evolution (e.g. Wright & Gribben 2008, Wright et al. 2012). The effects of NNS which are cryptic or old introductions are also difficult to study because the non-native status of the species is not clear (Mineur et al. 2014). In addition, impacts can vary depending on how abundant the NNS is (e.g. White & Shurin 2011). This means that studies of the NNS themselves (distribution, taxonomy, and abundance) are important for predicting and assessing impacts. For prediction, it is also important to understand how NNS abundance and distribution may be influenced by environmental changes. This is currently difficult, as there is still little understanding of the mechanisms controlling where or when a NNS becomes successful (Gederaas et al. 2012).

1.3 What determines non-native seaweed success?

It is first useful to define what success is in this context. I use it here as a term for the alga proceeding through the various stages of invasion in a new habitat (Fig. 2). It can thus refer to an NNS becoming established, more abundant, and/or more widespread. Three groups of factors may influence success: **propagule pressure**, **abiotic conditions**, and **community interactions** (the latter may also be called 'biotic conditions'; Colautti & MacIsaac 2004, Catford et al. 2009). These can influence success positively and negatively at various stages of the process (Fig. 2). Particularly in post-introduction stages, a NNS must not be limited by any of the three factor-groups: for it to establish at a new site or proliferate, a) propagules must be present, b) abiotic conditions must be suitable, and c) its settlement, recruitment, growth and survival must not be limited by community interactions. All are important, and in particular biotic interactions may have impacts on species distributions over larger scales than previously thought (Wisz et al. 2013). The same three criteria can also be applied to native species spread and proliferation, as the underlying processes are similar for native and established non-native species (Colautti & MacIsaac 2004).

These three factor-groups (propagule pressure, abiotic characteristics and community interactions) can thus be used to consider why some NNS are more successful than others. For example, a NNS which has wide salinity and temperature tolerances will be less limited by abiotic characteristics than a NNS with narrower limits, potentially allowing growth over a larger area; a NNS which produces many, widely dispersed propagules may be more likely to spread than one which disperses locally; a NNS that is a strong competitor and resists grazing may be able to establish in habitats where community interactions would inhibit other NNS. One might therefore expect successful NNS to conform to certain types, e.g. opportunistic species, or strong competitors (Valentine et al. 2007). However, there is not strong evidence of this in seaweeds, with the traits of successful NNS varying and often shared with natives and less successful NNS (Nyberg & Wallentinus 2005, Valentine et al. 2007). This suggests that there is no one strategy for success; some traits may compensate for others, or different traits may result in success in habitats with different conditions.
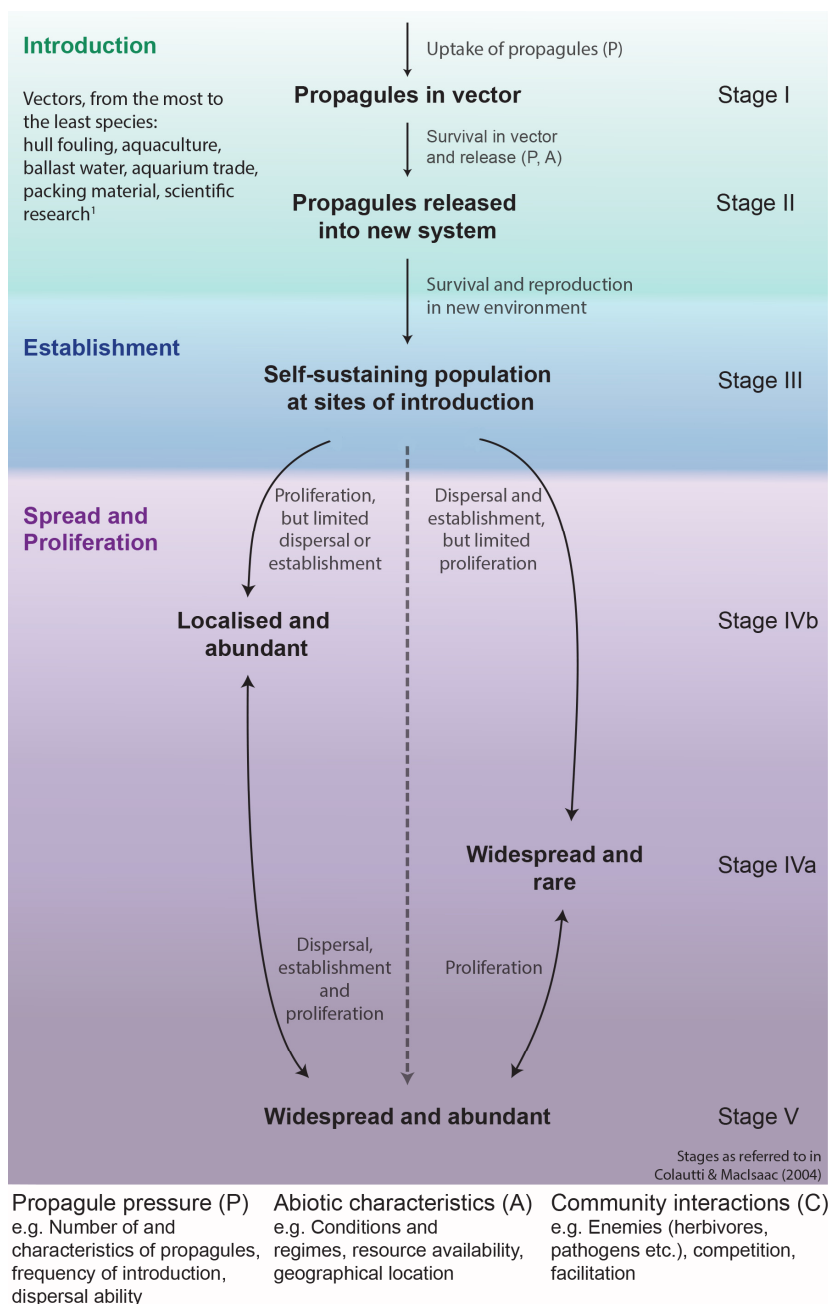
Figure 2 A framework to describe the stages of NNS invasion, adapted from Colautti & MacIsaac (2004) and Catford et al. (2009). Arrows have a short description of what must occur to progress. In the 'Introduction' stage, certain factors-groups are likely to be important (given in brackets); in all others, all three probably play a role (P, A, C). In the 'Spread and Proliferation' stage, the two routes represent relative extremes; a NNS could disperse and proliferate similarly (dotted path). On these two routes, the term 'establishment' refers to recruitment, growth and survival at new sites in the non-native range; it does not refer to the previous stage of invasion. [1]Hewitt et al. (2007)

Although the three factor-groups can be a useful way to think about the invasion process, individual hypotheses which call on specific mechanisms are often tested in success studies (for an overview, see Catford et al. 2009). These hypotheses can nonetheless generally be related to the three categories (Catford et al. 2009). For example, under propagule pressure there is the aptly named 'propagule pressure' hypothesis, which suggests that the more abundantly and frequently propagules are introduced, the higher the changes of success. This may be particularly relevant in early invasion stages as stochastic events can have a large influence (Simberloff 2009). The more times a NNS is introduced, the more propagules are released, or the more hardy its propagule, the better its odds. Additional propagules can also increase the genetic diversity of the population (Simberloff 2009). Many successful NNS are able to reproduce asexually or by selfing, and have propagules which can travel long distances (Valentine et al. 2007), supporting this idea. However, these characteristics do not apply to all successful NNS (e.g. Johnson et al. 2012).

Another example, under community interactions, is the 'enemy release hypothesis' which suggests that non-natives are successful in their new habitat because they are less supressed by natural enemies than native species (Keane & Crawley 2002). For NNS, this is supported by the fact that several NNS produce secondary metabolites which make them unappealing to herbivores (e.g. Lyons et al. 2007, Enge et al. 2013). However, native species may also produce these, and not all grazers are deterred by them (e.g. Thomsen & McGlathery 2007, Strong et al. 2009, Pedersen et al. 2016). In addition, the relatively low proportion of specialist grazers in the marine environment (Hay & Steinberg 1992) means that escape from specialist grazers is perhaps unlikely to be a common driver of success for seaweeds.

Again related to community interactions, the 'disturbance hypothesis' suggests that disturbance to the recipient community gives NNS the opportunity to invade (see Sher & Hyatt 1999). This is relatively common in systems where NNS are successful (Valentine et al. 2007). If competition from other seaweeds is limiting NNS success, disturbance can reduce this, but disturbance can also be thought of as an event that causes surplus resources, with this being the ultimate driver of success (Davis et al.

2000, Dunstan & Johnson 2007). Several invasion-success hypotheses are linked to this idea (Catford et al. 2009), which can be briefly summarised as: resources unused by the native community (space, light or nutrients, for seaweeds) give NNS a chance to establish/proliferate. Resources can be constantly available if the native community is naturally species-poor and does not use all resources ('empty niche hypothesis', MacArthur 1970), or fluctuate if the community is disturbed or if resources are added (e.g. nutrient enrichment) (Davis et al. 2000). It thus provides a unifying theory for several situations, including those where one non-native species facilitates establishment of another (Simberloff & Von Holle 1999). In that situation, resources may be available due the first non-native providing new resources (e.g. creating new habitat; Thomsen et al. 2010) or disturbing the community (e.g. Levin et al. 2002). This hypothesis also explains why a NNS may be successful in some places/times but not others (Johnson 2007). However, the importance of disturbance is not clear in all situations. It may not be necessary to maintain established NNS populations, and some species may establish without disturbance (Johnson 2007, Valentine et al. 2007, Morelissen et al. 2016).

If resource availability/disturbance *is* important for NNS success, it suggests that undisturbed native seaweed communities should be relatively invasion resistant. However, it also suggests that environmental changes will influence success. For example, if warming disturbs cold-adapted native species, community interactions with the NNS will change and more resources may become available. Furthermore, if warming improves abiotic conditions for growth and reproduction of the NNS, this could lead to changes in geographic range, abundance and competitive ability. Changes in propagule pressure may then occur as a result, or as a result of warming itself, such as changing current patterns.

1.4 Main objective

Environmental changes in the North Atlantic are expected to benefit non-native seaweeds (Brodie et al. 2014), and already there are examples of NNS flourishing in habitats affected by environmental change (e.g. Harris & Tyrrell 2001, Filbee-Dexter et al. 2016). However, for management purposes, predictions about specific NNS or specific habitats may be required. Whether the general expectation of increased NNS success with environmental change can be applied to individual NNS taxa or habitats is unclear, as different types of environmental change and factors affecting NNS success may combine together in complex ways and vary between taxa. Thus to predict how specific species or habitats may be influenced by environmental change, we need knowledge about that particular species and the area of interest.

In Norway *Codium fragile* subsp. *fragile* (Suringar) Hariot (Chlorophyta)*,* and *Sargassum muticum* (Yendo) Fensholt (Phaeophyceae) are two common non-native seaweeds. Along with *Bonnemaisonia hamifera* Hariot (Rhodophyta) and *Dasysiphonia japonica* (Yendo) H.-S.Kim (Rhodophyta), these are the seaweeds ranked in severe or high impact categories in the Norwegian blacklist of alien species (Gederaas et al. 2012). As *B. hamifera* is a small understorey species and *D. japonica* is a filamentous sublittoral alga, the abundant canopy-forming species *C. fragile* subsp. *fragile* and *S. muticum* were chosen as the focus of this project. Both are already widespread (stages IVa to V in Fig. 2) and can form dominant canopies in low littoral to upper sublittoral tidal zones in southwestern Norway.

The main objective of this project was to study how selected abiotic and biotic factors affect the success of *Codium fragile* subsp. *fragile* and *Sargassum muticum*, and to investigate whether environmental changes will benefit these species in southwestern Norway. For factors which may limit success, I have focused on substratum, wave-exposure, and community interactions with native canopy seaweeds. For the impact of environmental changes on success, I have focused on temperature and nutrient enrichment. Specific research questions to address this objective were developed based on existing knowledge of the study area and focal species, which are introduced in the following section.

1.5 Study system

1.5.1 Field study area

This fieldwork of this project was mostly restricted to the southwestern coast of Norway, among the islands and fjords outside Bergen (Fig. 3). No work was done on the Atlantic-facing shores of outer islands, which are very wave-exposed and thus mostly unsuitable for the target species of this project. The substratum in the littoral and upper sublittoral zones is mostly hard, either stable bedrock or loose boulder/cobble substratum (I refer to the latter as 'stony'). The inclination of bedrock can vary from vertical walls to gentle slopes. The large number of islands and abundance of hard substratum mean that there is a huge area of potential habitat for canopy-forming seaweeds in this region, and the coast of Norway in general.

► **Figure 3** Map of the study area. Map A shows southern Norway and the area enlarged in map B. Map B shows the main study area, and the location of two hydrographic stations (*black diamonds*) from which temperature and salinity data was extracted, with Indre Utsira to the south and Sognesjøen to the north (both run by the Institute of Marine Research). Bergen and Espegrend Marine Biological Station are shown for reference. *Figure modified from **Paper III**.*
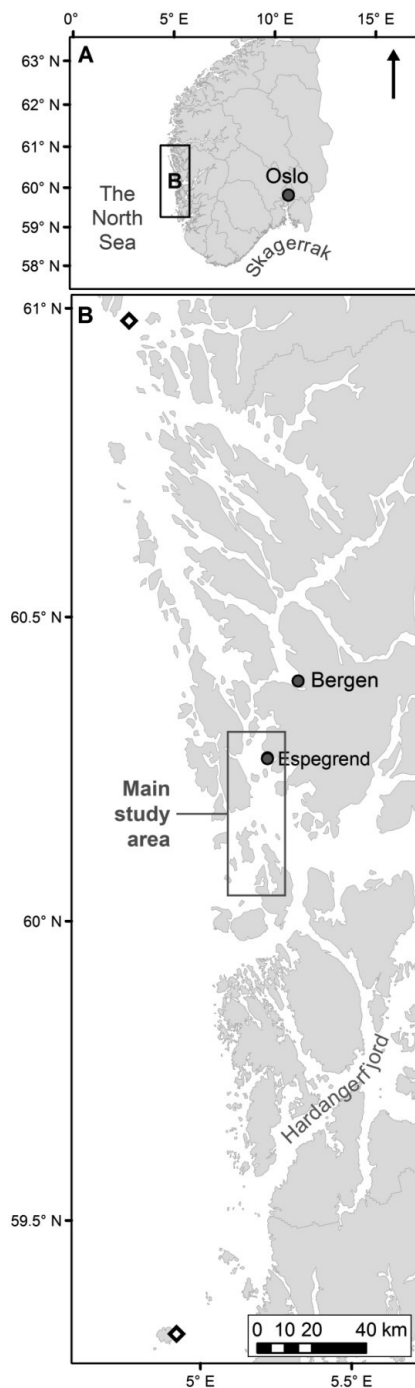
According to the definitions of Lüning (1990), the study area is in the cold-temperate region (northern Norway to northern France). Sea surface temperatures are highest in August and lowest in February/March, with the last 10 years (2007-2016) having an August mean of 16.5 °C and February/March mean of 5.1 °C (Institute of Marine Research 2017; Fig. 4). Temperature series show that seawater temperatures have been increasing since 1990 relative to the normal along the Norwegian coast, with a 0.5 °C increase in the deeper ocean layers attributable to global warming (Bakketeig et al. 2016). Surface water is more affected by weather conditions than deep water, but also indicates that the last two decades have been warm, particularly in summer (Fig. 4). Under a moderate greenhouse gas emission scenario, sea surface temperatures in 2080-2099 are expected to be 1.5-2 °C higher in February and 2-2.5 °C higher in August than they were 1980-1999 in the region (Müller et al. 2009).
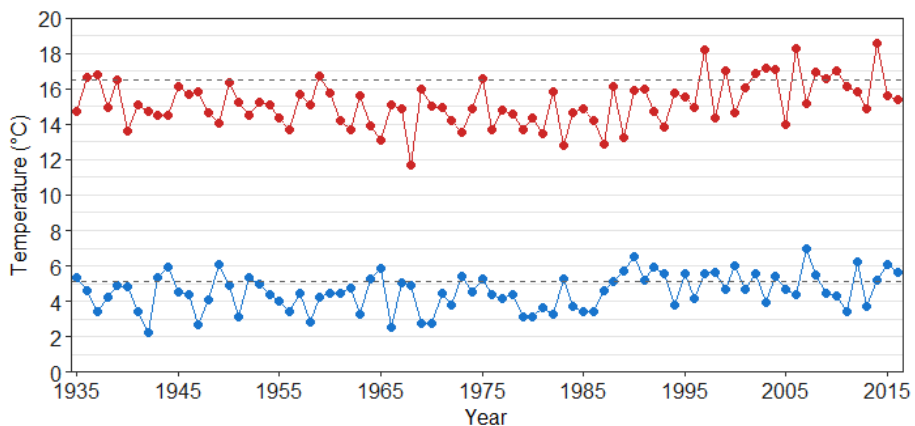
Figure 4 August (*red*) and February/March (*blue*) mean temperatures at 1 m depth at Indre Utsira and Sognesjøen fixed hydrographic stations (locations shown in Fig. 3; Institute of Marine Research 2017). The dotted lines show the means of the respective months in the last decade (2007-2016).

Salinity in the area is slightly lower than full seawater, ranging from around 32 in winter/spring to around 28 in summer (1987-2016 monthly means at Indre Utsira and Sognesjøen; Institute of Marine Research 2017). Irradiance is strongly seasonal, peaking in June, while nutrients in seawater (nitrate, ammonium and phosphate) show the opposite pattern, and are lowest in late spring/summer. Peak nutrients, light and temperature are therefore asynchronous in the area (Fig. 5).
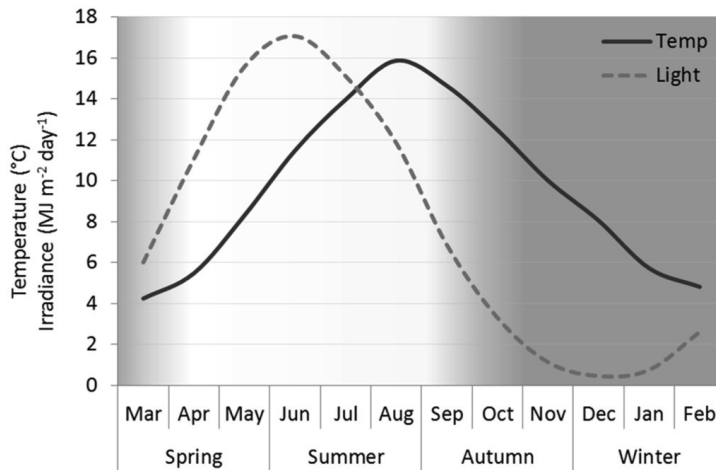
Figure 5 Seasonal cycles of light, temperature and nutrients in southwest Norway. Temperature data is from Indre Utsira hydrographic station ("Temp", 1980-2016 monthly means; Institute of Marine Research 2017). Irradiance data is from Bergen, measured as the global daily irradiance at the surface ("Light" , 1963-2013 monthly means; GFI, University of Bergen). Relative nutrient levels are shown by grey shading, where the gradient of white to grey corresponds to low to high nutrient levels (data from Strömgren 1986, Pedersen & Borum 1996). *Figure from **Paper III**.*

Mean sea level in the area is around 90 cm (Kartverket 2017). The shore is usually vertically divided into the supralittoral (almost never submersed, above high spring tides), the eulittoral (periodically submersed, between low and high tides) and the sublittoral (almost never emersed, except the uppermost part at strong low tides) (Lüning 1990). However, the present project concerns species which have a sublittoral or low littoral distribution, so I subdivide the lower sections of the shore. I refer to the shore above mean low water (MLW, ca. 45 cm) as the littoral, between MLW and mean low spring water (MLSW, ca. 30 cm) as the low littoral, between MLSW and chart datum (CD, 0 cm) as infralittoral, and below CD as sublittoral (heights from Kartverket 2017). Under this system, the littoral is emersed almost daily, the low littoral emersed regularly, the infralittoral emersed rarely, and the sublittoral emersed almost never.

In this project I have focussed on fleshy canopy-forming algae in sheltered to semi-exposed habitats. In this region, the most likely relevant environmental changes affecting these are probably temperature changes and nutrient enrichment (Moy & Christie 2012, Gundersen et al. 2016). Large urchin barrens are not present in the

study area, beginning further north in Norway (Norderhaug & Christie 2009) and these shores do not bear the brunt of storms. Increasing carbon dioxide concentration could influence the region, through an effect on canopy algae, grazer-interactions, and even non-native success (Arnold et al. 2012, Asnaghi et al. 2013, Olabarria et al. 2013, Strain et al. 2014). However, this factor was outside the scope of the project and is not considered further.

As southwestern Norway is relatively far north in the temperate region, one might not expect obvious changes to distributions of temperate seaweeds with small increases in temperature. However, effects have already been documented in southern and southwestern Norway. Warming has been associated with changes in community composition (Sjøtun et al. 2015), and increases in southern seaweed species (Husa et al. 2008), while extremely hot summers can cause death of littoral seaweeds (Husa et al. 2007) and have been linked to losses of the kelp *Saccharina latissima* (Moy & Christie 2012).

Moy & Christie (2012) also suggested that nutrient enrichment might have been involved in the decrease in *S. latissima* abundance which took place in the early 2000s. Eutrophication tends to have negative effects on canopy algae, favouring ephemeral, filamentous or turf-forming species (Worm et al. 2000, Bokn et al. 2002, Strain et al. 2014), and even short, sporadic nutrient pulses can lead to increased growth of filamentous epiphytes on canopy seaweeds (Worm & Sommer 2000). When these grow on kelps, they can reduce available light and increase mortality (Andersen et al. 2011). Although the southwest coast of Norway is relatively unaffected by nutrient enrichment compared to the Skagerrak area (Aure et al. 1996), eutrophication is classed as a threat to kelp in this area by some (Gundersen et al. 2016). Local nutrient enrichment may occur as a result of aquaculture, with Hordaland having the highest per-area nutrient inputs in the country. The overall effect of this appears to be relatively small, but seaweeds in the vicinity of farms could be subject to higher nutrients, the effects of which have not been assessed (Taranger et al. 2011).

1.5.2 Study species

Native species in the study area

Substratum in the low-littoral to upper-sublittoral is usually colonised by canopy-forming kelps and fucoids, beneath which is variable cover of turf (here defined as perennial, substratum-occupying species ca. <15 cm high) and encrusting algae (Fig. 6). However, small areas of substratum without canopy cover, or with canopy but without perennial turf, are not uncommon. Kelp and fucoid species differ in their form, life history, and preferences for wave-exposure and tidal height (Table 1). They are therefore likely to differ in vulnerability to temperature and nutrient changes. Infralittoral kelps such as *Laminaria digitata* and *Saccharina latissima* may be most at-risk from temperature increases in southern Norway, given that they lack the stress-adaptations and temperature tolerances of littoral fucoids (Table 1; Lüning 1984), but may still be exposed at very low tides and are close to the surface where heatwaves increase water temperature (e.g. as observed by Hawkins & Hartnoll 1985). Declines of *S. latissima* have already been associated with warming in cold temperate areas (Moy & Christie 2012, Filbee-Dexter et al. 2016). If interactions between native species and NNS control NNS success, negative impacts on these species could lead to an increase in NNS success.

There are several other canopy species which occur in the area but are not discussed, either because they are outside the depth/exposure ranges of focal species in this project or because they are not often dominant. These include *Laminaria hyperborea* (mostly deeper and at more exposed sites; Kain 1979), *Alaria esculenta* (only at wave-exposed sites), *Fucus spiralis* and *F. vesiculosus* (can form a zone in the mid-littoral, but do not occupy large areas at most sites), *Desmarestia* spp. and *Halidrys siliquosa* (can grow in the upper sublittoral but usually as scattered individuals), and *Sacchoriza polyschides* (an annual sublittoral kelp, relatively uncommon) (pers. obs. and Rueness 1977). The long, thread kelp *Chorda filum* can often be found in the area in the upper sublittoral, but is only common in summer, and at densities which would not be expected have a strong competitive effect on algae below.
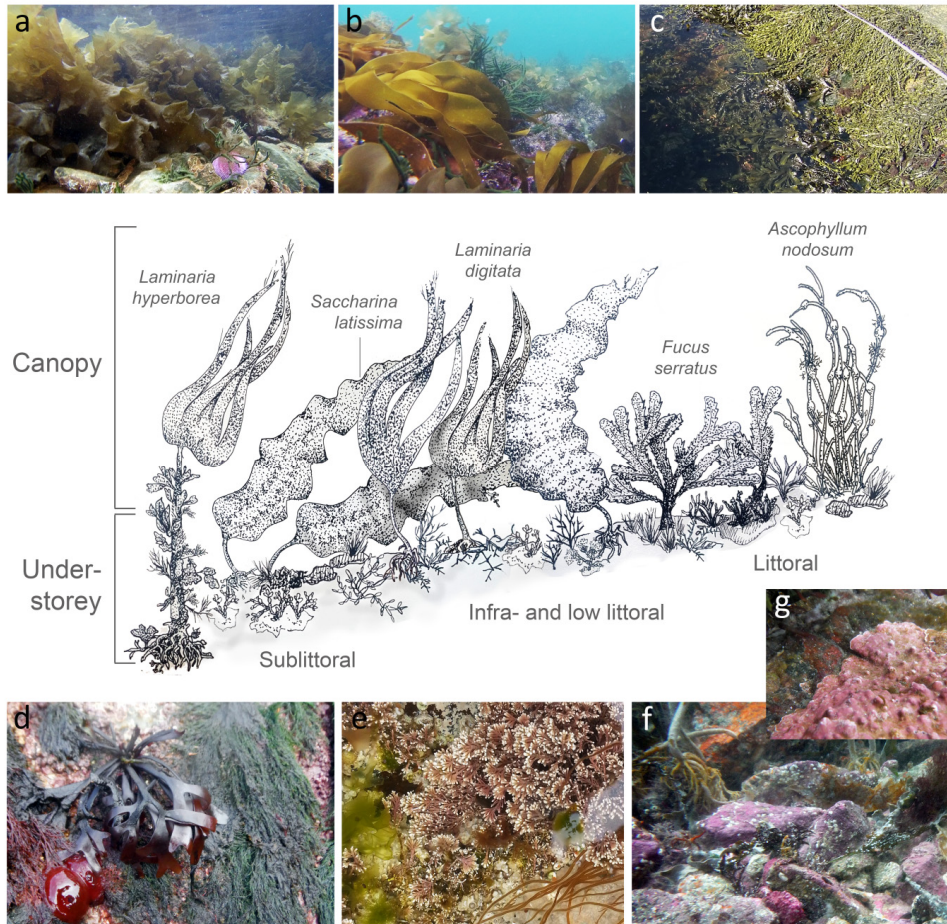
Figure 6 Schematic illustration of vegetation structure on hard substratum in the sublittoral-littoral in southwestern Norway, with photos. Native canopies consist mostly of fucoids and kelps, with an understory of perennial turf species, ephemeral and epiphytic algae (although the latter two may also grow in the canopy itself). Which canopy species become dominant depends on depth, exposure and substratum, partly due to the influence of these factors on competition. Note that although the labelled species are all termed "canopy species", they may become understorey species in certain situations, e.g. if the flexible *Laminaria digitata* grows alongside the long, stiff-stiped *L. hyperborea* (Kain 1979) or when juveniles. Sizes of thalli may vary considerably compared to the drawing. **Photos:** (a) A canopy of *Saccharina latissima*, with long blades lying over the substratum; (b) *Laminaria digitata* in the infralittoral, with understorey coralline turf algae; (c) a littoral *Ascophyllum nodosum* zone, with a *Fucus serratus* zone deeper; (d) common perennial turf species *Cladophora rupestris* (green filamentous) and *Chondrus crispus* (red branched) in the littoral; (e) the turf-forming coralline alga *Corallina officinalis*; (f) coralline encrusting algae on stones beneath kelp stipes in the infralittoral zone; (g) a close-up photo of a coralline crust.

Table 1 Characteristics of some common large canopy-forming native species in the study area, which may grow at similar depths to the focal NNS. *Table modified from **Paper IV**.*

Species	Size and form[1]	Lifespan	Life cycle and reproduction	Preferred habitat[1]	Growth/Survival temperatures[2]
Fucus serratus Linnaeus	Bushy with flat leathery branches, 30-60 (-100) cm	Perennial, usually to 3 yr (Knight & Parke 1950).	Sexual, dioecious. Timing varies; often in winter/spring (Rueness 1977, Strömgren 1986), also in summer (Knight & Parke 1950). Low dispersal (Arrontes 1993)	Sheltered to exposed, low littoral/upper sublittoral	Optimum growth at 15°C, death at >25°C
Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders	Holdfast, stipe and lamina (1-3 m long, 10-30 cm wide)	Perennial, to 3 yr (Parke 1948). Lamina reduced via erosion	Sexual, alternation of generations. Timing varies but spore production most common in winter/early spring (Parke 1948)	Sheltered to semi-exposed. Sublittoral (1-30 m). On unstable substratum and sheltered stable substratum (Kain 1979)	Optimum growth at 10-15°C, rapid decrease and tissue damage at ≥18-20°C. Sporophyte death at >20°C, gametophyte death at >23°C
Laminaria digitata (Hudson) J.V. Lamouroux	Holdfast, stipe (2-3 m) and split lamina (to 1 m)	Perennial to 7 yr (Olsen 2004). Lamina reduced seasonally	Sexual, alternation of generations. *L. digitata* produces most spores in autumn although may also at other times in the year (Kain 1979)	Semi-exposed to exposed. Upper sublittoral.	Optimum growth at 10°C. Tissue damage at ≥18°C. Sporophyte death at ≥18-20°C , gametophyte death at >21°C

[1] From Rueness (1977) unless otherwise cited
[2] From Fortes & Lüning (1980) and Lüning (1990), with Andersen et al. (2013) for *S. latissima*, and Bolton & Lüning (1982) and Simonson et al. (2015b) for *S. latissima* and *L. digitata*

Codium fragile subsp. *fragile*

Codium fragile subsp. *fragile* (hereafter referred to as *C. fragile*, unless specified otherwise) is one of the top 10 non-native species in Europe in terms of impacts (Vilà et al. 2010), and one of the most prominent and well-studied NNS worldwide (Trowbridge 1998, Schaffelke & Hewitt 2007). In new habitats it may affect detritus cycling (Krumhansl & Scheibling 2012b) and the composition of fauna and epiphytic algae, although usually without negative impacts on diversity or richness (Schmidt & Scheibling 2006, 2007, Jones & Thornber 2010, Drouin et al. 2011, Armitage & Sjøtun 2016). It can also negatively affect aquaculture by growing on or amongst commercial species (Trowbridge 1998, Neill et al. 2006).

Codium fragile is now present on most continents (Guiry & Guiry 2017). In Norway it can be found along much of the coast, being absent only north of Tromsø (Stellander 1969), and rare in the southeast and around fjords with large river inputs (Fægri & Moss 1952, Husa et al. 2013). It is native to the NW Pacific and came to Europe prior to 1845, at first undetected due to its similarity to native subspecies (Silva 1955, Provan et al. 2005, Provan et al. 2008). The first Norwegian record is from 1946 (Silva 1957), but a large increase in *Codium* in the 1930s (Fægri & Moss 1952) suggests an earlier arrival. Although *C. fragile* is relatively common in western Norway, little work has been done on it here since the 1950s when its geographical distribution was mapped by Moss (1952). His herbarium samples were later examined by Silva (1957) in his taxonomic work on Scandinavian *Codium*.

The seaweed itself is a spongy, canopy-forming, branched alga to 50 cm, with a mat-like holdfast, and a siphonous structure consisting of tangled filaments (Fig. 7) (Rueness 1977, Brodie et al. 2007). It reproduces asexually via parthenogenetic gametes and fragmentation, and is buoyant in good light, allowing fragments to float and disperse (Gagnon et al. 2014; see **Paper III** introduction for a detailed description of reproduction). *Codium fragile* is perennial, but thallus fragmentation (Fig. 7) can reduce its length (Fralick & Mathieson 1972). Trowbridge (1998) provides an extensive review of the biology of this species, in addition to the information contained in the introductions of **Papers I-IV**.
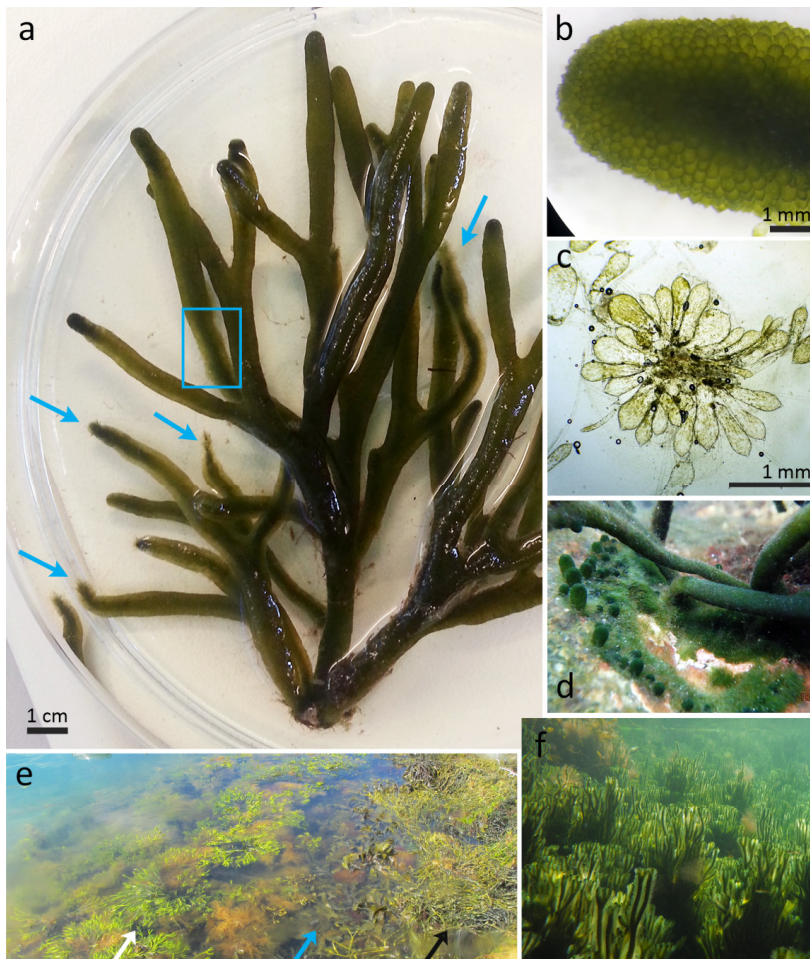
Figure 7 *Codium fragile* subsp. *fragile* thallus structure and habit in southwest Norway. (a) A thallus collected in October, when many branch tips are intact, but several (marked with arrows) and a section lower down (rectangle) have begun to unravel ("fragmentation"). (b) A magnified branch tip showing the surface structure, which is formed by the swollen ends of filaments ("utricles"). (c) A magnified cross-section of part of a branch, showing some of the central structure with utricles arranged to the outside. (d) A mat-like 'holdfast' (ca. 10 cm) from which upright branches are growing. (e) An infralittoral-sublittoral *C. fragile* zone with buoyant branches (white arrow). Higher on the shore is a zone of *Fucus serratus* (blue arrow) and *Ascophyllum nodosum* (black arrow). (f) A dense canopy of *C. fragile* in the upper sublittoral zone. *Figure modified from **Papers I and III**.*

Codium fragile is able to produce propagules and disperse easily, which likely contributes to its success. It also has relatively wide abiotic tolerances in terms of temperature, salinity (Hanisak 1979a) and shade (Thomsen & McGlathery 2007), and can recover from desiccation (Schaffelke & Deane 2005). In addition, it seems grow fairly well in low nitrogen. Although laboratory experiments by Hanisak (1979b)

suggest that its growth may be nitrogen limited, *C. fragile* can store some nitrogen in winter for use in spring (Hanisak 1979b) and may have other mechanisms to avoid limitation, such as nitrogen fixing-bacteria or strong nitrogen-scavenging abilities (Head & Carpenter 1975, Hanisak & Harlin 1978). It may however be limited by wave-exposure (Bulleri & Airoldi 2005), and by low temperature in cold-temperate regions (see **Paper III** introduction). Compared to native canopy species (Table 1), its optimum growth temperature is relatively high (24 °C) and it can survive to at least 30 °C (Hanisak 1979a, Lüning 1984), suggesting that warming may benefit *C. fragile* in Norway.

There have been several studies of interactions between *C. fragile* and native macrophytes. Some indicate that disturbance of native canopy species may increase *C. fragile* success (Levin et al. 2002, Scheibling & Gagnon 2006, Gagnon et al. 2014), with the alga often successful on artificial structures (Bulleri & Airoldi 2005, Neill et al. 2006, Geraldi et al. 2014). This also applies to its native region, where *C. fragile* is rarely dominant in the absence of disturbance (Chavanich et al. 2006). However, *C. fragile* success can also be positively related to native macrophyte density (Drouin et al. 2016), and mussels may facilitate *C. fragile* under some conditions (but inhibit it in others; Bulleri & Airoldi 2005). This indicates that the impacts of disturbance can be situation-specific. Pre-emptive competition between native canopy species and *C. fragile* is probably important, with established *C. fragile* able to inhibit re-establishment of kelp (Levin et al. 2002, Scheibling & Gagnon 2006). However, Watanabe et al. (2010) found declines in *C. fragile* and increases in kelp cover over time, suggesting that prevention of kelp establishment is a short-term effect. In terms of non-preemptive competition, *C. fragile* does not affect kelp growth (Levin et al. 2002), and is an inferior competitor to seagrass (Malinowski 1974). Drouin et al. (2012) found negative effects of *C. fragile* on seagrass in experiments, but these did not result in detectable effects in nature. *C. fragile* has also failed to replace native *Codium* in southern England (Trowbridge & Farnham 2009).

In summary, *C. fragile* appears to have relatively small competitive effects on other seaweeds in terms of interference/exploitative competition, but can have a negative

effect through pre-emptive competition. It itself may be limited by competition, but may also be facilitated by native species in some habitats. This provides some insight into factors affecting *C. fragile* success, but several questions remain. It is unclear how context changes the effect of disturbance, and how long pre-emptive occupation of substratum by *C. fragile* lasts, with studies into longer-term trends in abundance of *C. fragile* only done in small parts of its range (Watanabe et al. 2010, Filbee-Dexter et al. 2016, Trowbridge et al. 2016). In general there is a geographic bias in work on *C. fragile*, with most studies (particularly success experiments) done in the NW Atlantic, with a few observational studies from the British Isles. Basic information about *C. fragile*, or its distribution, is lacking for Norway.

Not only is this important from a management perspective, but may also be important for success studies. Southwestern Norway has a complex coastline with variation in wave-exposure and hard substratum types over small spatial scales. Although *C. fragile* is not successful under high wave-exposure, it is unclear how this factor or different types of hard substratum affect its success within 'tolerable' levels. Native communities in the NE and NW Atlantic also differ, with *Fucus serratus* native only in the NE. If community interactions limit *C. fragile*, this species may affect its success here. In addition, generalised descriptions of *C. fragile* distribution are sometimes used when discussing success: in the NE Atlantic it may be described as a mostly littoral, low abundance species which does not monopolise space, in contrast to a sublittoral, dominant species in the NW Atlantic (e.g. Chapman 1999, Mathieson 2003, Schaffelke & Hewitt 2007). These NE Atlantic descriptions are based on the British Isles. However, older sources from Norway describe a sublittoral distribution where *C. fragile* can be locally abundant (Sundene 1953, Jorde 1966), and occur instead of native assemblages (Jorde 1966). If *C. fragile* in the NE Atlantic varies in success and habit, this has implications for any insights into success based on NE vs. NW contrasts, and can be clarified by further work on the species in Norway.

Location of study also relates to the question of how *C. fragile* might be affected by warming. Effects of temperature on *C. fragile* are relatively well-studied, but again most work in its non-native range is from the NW Atlantic (e.g. Churchill & Moeller

1972, Fralick & Mathieson 1972, Fralick & Mathieson 1973, Malinowski 1974, Hanisak 1979a, Bégin & Scheibling 2003, Lyons et al. 2010, Wilson et al. 2015; but see Malinowski 1974, Yang et al. 1997, Madariaga et al. 2014). However, there are indications that its temperature optima may vary depending on location (Malinowski 1974, Trowbridge 1998). If *C. fragile* has adapted or acclimatised to local temperature regimes, the effects of warming may vary between regions, resulting in different outcomes for success.

Finally, there are some taxonomic questions for this species. Subsp. *fragile* is the only subspecies of *C. fragile* which has spread extensively as a NNS, with 9 other subspecies locally distributed around the world (Brodie et al. 2007). In Norway, subspecies *fragile, atlanticum* and "*scandinavicum*" have been recorded (Silva 1957). However, Provan et al. (2008) found that these subspecies have been frequently misidentified historically, and that "subsp. *scandinavicum*" is the same as subsp. *fragile* based on sequencing of the type specimen. That study did much to clarify the subspecies situation, but only one sample was included from Norway (which was the non-native subspecies). Therefore it is uncertain whether subsp. *atlanticum* or another northern subspecies are actually present in Norway. Confident identification is critical for being able to study the success and impacts of the non-native subspecies, especially as *C. fragile* subspecies can differ in ecological function (Lutz et al. 2010).

Sargassum muticum

Sargassum muticum is another relatively well-studied NNS (Thomsen et al. 2016). Once established, it may alter detritus cycling (Pedersen et al. 2005), food-webs (Salvaterra et al. 2013), and the composition of seaweed-associated communities of flora and fauna, although strong negative impacts on fauna are not reported (Viejo 1999, Wernberg et al. 2004, Buschbaum et al. 2006, Harries et al. 2007, Gestoso et al. 2012, Engelen et al. 2013). As probably the case for NNS generally, its effects on native species may vary by habitat and its abundance (Buschbaum et al. 2006, Lang & Buschbaum 2010, White & Shurin 2011).

Sargassum muticum is a canopy alga which may reach several metres in length in some locations (Engelen et al. 2015), but in Norway mostly remains below 1 m (Gederaas et al. 2012). Its canopy is buoyant due to air vesicles, but is also ephemeral here, as the branches ("laterals") of *S. muticum* regrow from a small perennial basal part each spring and are lost in early autumn (Fig. 8). It has a rapid growth rate to achieve such lengths within this period (Norton 1977a). Because of this cycle, *S. muticum* may be referred to as pseudo-perennial. It is monoecious and reproduces via gametes, but produces many and has the ability to self-fertilise (Norton 1981, Engelen et al. 2015). Its germlings only disperse very short distances from the parent thallus, but laterals begin to break off when reproductive and can float, allowing the dispersal of germlings over longer distances (Norton 1977a, Deysher & Norton 1982, Kendrick & Walker 1995, Engelen et al. 2015). There are reports of drift fragments arriving in areas before the establishment of attached thalli (Rueness 1989), suggesting that this ability contributes to its success. Engelen et al. (2015) provide a detailed review of the biology, ecology and history of this species.

Figure 8 *Sargassum muticum* thalli and habit in southwestern Norway. (a) A patch of *S. muticum* in the infralittoral-upper sublittoral zone, showing the dense buoyant canopy (*Photo: Mette Eilertsen*) (b) A small *S. muticum* individual, as one might find in early April, ca. 20 cm long (*Photo: Kjersti Sjøtun*). (c) A *S. muticum* thallus in late July, attached to a piece of rope at the base (ca. 80 cm long). *Figure modified from **Paper V**.*

Sargassum muticum has spread extensively in the NE Pacific and NE Atlantic from its native range in the NW Pacific (Engelen et al. 2015) and reached Norway around 1988 (Rueness 1989). Since then it has spread along the south and southwest coasts, north to at least Molde (62.8 °N). Southwards along the NE Atlantic coast, its range stretches into Tunisia (Engelen et al. 2015). This distribution reflects the fact that *S. muticum* has relatively wide abiotic tolerances to temperature, and some tolerance to low salinity (Norton 1977a, Norton 1977b). It can grow from at least 5-25 °C, with faster growth and higher germling survival at upper end of that range (Norton 1977a, Steen & Rueness 2004). This, combined with its southern distribution, suggests that warming in Norway might make *S. muticum* more successful.

The fast growth and buoyant laterals of *S. muticum* make it an effective competitor for light, and it may shade other seaweeds when it grows in the infralittoral or sublittoral (Ambrose & Nelson 1982, Britton-Simmons 2004). Due to its pseudo-perennial life cycle, its ability to compete via exploitation is probably higher than its ability to compete via pre-emption, except perhaps where native species recruit during its period of peak biomass (e.g. Ambrose & Nelson 1982). Decreases in abundances of native seaweeds have been observed with increases in *S. muticum* (e.g. Stæhr et al. 2000, Britton-Simmons 2004, Harries et al. 2007). Conversely, community interactions with native seaweeds may also limit *S. muticum.* Successful recruitment can be limited in several ways: native canopies can shade juvenile *S. muticum* (Britton-Simmons 2006, Vaz-Pinto et al. 2012) or prevent germlings reaching substratum (Sánchez & Fernández 2006), while turf-forming species lower the availability of space for recruitment to occur (Britton-Simmons 2006, although this was not found by Vaz-Pinto et al. 2012). The perennial holdfast of *S. muticum* is an advantage in this situation, as it allows regrowth without new recruitment. While effects of *S. muticum* on other species (and vice versa) in the littoral zone are variable (see **Paper V** introduction, and Viejo 1997), it appears that competition for light or space could be an important determinant of success for this species in the infra- and sublittoral (Deysher & Norton 1982).

Relatively many success studies have been done with *S. muticum*, and it is one of the few NNS where interactive effects have been tested. For example, disturbance or manipulation of seaweed communities with varying propagule pressure (Andrew & Viejo 1998, Britton-Simmons & Abbott 2008, Vaz-Pinto et al. 2012), disturbance with nutrient enrichment (Sánchez & Fernández 2006, Bertocci et al. 2015), grazing with nutrient enrichment (Vaz-Pinto et al. 2013a), temperature and CO_2 increase (Vaz-Pinto et al. 2013b), and the effects of different functional groups (Deysher & Norton 1982, Britton-Simmons 2006). These indicate that propagule pressure is an important factor in invasion success, and that disturbance of native species can facilitate *S. muticum*, especially when propagule pressure is high. Grazer-interactions could also play a role, as herbivores can graze on *S. muticum* and reduce survival of recruits (Sjøtun et al. 2007, Vaz-Pinto et al. 2013a). Although some herbivores prefer native seaweeds (Monteiro et al. 2009), enemy-release is not likely to be the mechanism behind this alga's success (Pedersen et al. 2016).

Competition may be important for the success of *S. muticum*, but few studies have included the common canopy species which it might compete with in Norway, *Saccharina latissima* and *Fucus serratus*. Stæhr et al. (2000) documented reductions in *Saccharina latissima* and *F. serratus* with *Sargassum muticum* invasion, but Strong and Dring (2011) found no negative competitive effects on *Saccharina latissima* in experiments. This leaves questions about how it interacts with these seaweeds, and how disturbance of them might affect its abundance. The role of nutrient levels in the success of *Sargassum muticum* is also unclear. Studies may show no impact of nutrient enrichment (Bertocci et al. 2015), positive effects (Sánchez & Fernández 2006), or concentration-dependent impacts, where some enrichment increases initial recruitment, but too much increases growth of ephemeral algae which occupy substratum instead (Vaz-Pinto et al. 2013a). In addition, most success studies have been done in southern locations, and it is possible that the factors controlling *S. muticum* success could be different in Norway, where there are different competitors, lower temperatures, more extreme seasonal differences in day-length, and very low summer nutrients.

1.6 Research questions

As outlined previously, there are many factors which potentially affect NNS success. Therefore I examined a small subset which were practically possible for me to study during this project. As before, *Codium fragile* refers to subsp. *fragile*, unless otherwise specified. The research questions were as follows:

1) *How long has the non-native* C. fragile *been in Norway, and are there other subspecies present in the study area?*

    This question is not related to the main aim of the project, but I consider it critical background work for ensuring that the conclusions of subsequent studies are valid. I sequenced DNA from fresh and herbarium samples of *C. fragile* (of unknown or morphologically determined subspecies identity) from along the coast to identify subspecies. I then compared morphology with genetic identity to establish the reliability of commonly-used subspecies traits (**Paper I**).

Abiotic and biotic factors influencing success

2) *Are substratum type and wave-exposure related to the abundance and local distribution of* C. fragile *in sheltered to semi-exposed habitats?*

    To address this question I conducted a survey of sites which had varying hard substratum types, wave-exposure, and abundances of *C. fragile.* In the first survey (**Paper II**), I recorded the vertical range and abundance of *C. fragile*, and looked for relationships with substratum and wave-exposure. I carried out subsequent abundance surveys at the same sites (**Paper III**) to see whether the association with substratum was maintained. As field surveys were used, the conclusions are based on associations, rather than demonstrated causation. Abundance was recorded by estimating the number of thalli at a site (in categories), and combining it with their distribution at the site (individual/patch/zone).

3) *How persistent are established* C. fragile *populations, and is this related to substratum?*

    To answer this I carried out repeated surveys at the stations from Paper II to track changes in the abundance of *C. fragile* over 5 years (**Paper III**).

4) *Is there evidence that* C. fragile *competes with* Fucus serratus*?*

I addressed this by recording the vertical range and abundance of *C. fragile* and *F. serratus* at field survey sites, and looking at the relationship between them and abiotic factors (**Paper II**). This methodology cannot demonstrate competition, but can show whether distribution patterns consistent with competition are present.

5) *Does canopy-disturbance benefit* C. fragile *(or* Sargassum muticum*)?*

I tested this by carrying out a field experiment where seaweed cover was measured in treatment and control transects (canopy-clearance vs. un-manipulated) over 2-3 years, allowing the effect of canopy-disturbance on cover to be established (**Paper IV**). As both species are present in the study area, both were included in the observations (as was *Bonnemaisonia hamifera*); however, *C. fragile* was the focal species as it is most abundant, thus sites were chosen based on its presence. The experiment tested for an effect of disturbance, but did not examine the potential mechanisms of competition or resource availability.

6) *What are the competitive relationships in canopies of* Sargassum muticum, F. serratus *and* Saccharina latissima *in the upper sublittoral?*

I created fixed-density canopies of these three species in various combinations, using thalli of the same length. I then compared the growth and survival of the species between the assemblage types over summer in the field (**Paper V**). The experimental manipulation allowed competitive effects to be demonstrated, but because it was done in the field, the possibility of other factors interfering cannot be excluded.

Environmental change and success

7) *How is the growth rate of* C. fragile *from southwestern Norway related to temperature, and will future warming increase its growth rate?*

I used a combination of experimental and observational approaches to investigate this. I measured growth rates at recent and future temperatures (+2-3 °C) under laboratory conditions. This excluded any confounding factors which can influence results in the field, allowing a causal effect of temperature on growth to be

established, and a comparison of growth rates with experiments in other locations. However, laboratory conditions exclude factors that may affect growth rates in nature, therefore I also recorded growth in the field to measure the *in situ* association with temperature (**Paper III**).

8) *Are changes in* C. fragile *local distribution patterns related to temperature?*

I used the results of the surveys carried out for RQ3 to see if local abundance was related to measured sea surface temperatures during the 5 year period (**Paper III**).

9) *Are competitive relationships in canopies of* Sargassum muticum, F. serratus *and* Saccharina latissima *likely to change with hotter summers?*

I tested the effect of temperature by carrying out the experiment for RQ6 once in a 'normal' summer, and once in an unusually hot summer (**Paper V**). Because the effect of temperature was measured over two different years in the field, I cannot exclude that conditions in the first year could have affected conditions in the following year. The limitations of this experiment are discussed in Paper V.

10) *Does nutrient enrichment benefit* Sargassum muticum*?*

Observations led us to suspect that *S. muticum* might be suffering from nutrient limitation in late summer (described in Chapter 2). In order to test this, I did a field-experiment taking advantage of a nutrient-emitting industry on the coast of Norway, salmon farming. I placed *S. muticum* next to two farms and at three reference sites, and compared the growth, condition, and nitrogen content of the thalli. I have included these results because they are relevant to the aim and could be useful for further studies; however, they do not comprise a whole study and are thus not in manuscript format. Instead I have written a description of the methods in Appendix 4.1, and refer to the results as **Study I** in Chapter 2 to indicate where the data come from.

2. Results and Discussion

2.1 Basic information documented

During the project I collected some basic information about the non-native seaweeds which may be useful for future studies or as a reference for comparison with other locations. I have summarised this here before the addressing the research questions.

Codium fragile

Both subsp. *fragile* and subsp. *atlanticum* may be found in Norway, with subsp. *fragile* present since at least 1932 (**Paper I**). This fits with observations of a rapid increase in *C. fragile* in the 1930s (Fægri & Moss 1952). My results indicate that subsp. *fragile* currently accounts for the vast majority of *C. fragile* at sheltered to semi-exposed sites in southwestern Norway, with no contemporary subsp. *atlanticum* found south of Trøndelag. However, I suspect that if extensive studies of outer, exposed islands were conducted, there might be rare populations of subsp. *atlanticum* in southwestern Norway given its presence in Scotland (Trowbridge & Todd 1999a) and a historical specimen from Sogn og Fjordane (**Paper I**). Subsp. *atlanticum* was not found sublittorally, and only in one area, thus may have a more restricted habitat and distribution than subsp. *fragile* (Fig. 9). However, additional surveys are needed to assess this properly.

Subsp. *fragile* and subsp. *atlanticum* can be similar in morphological characters traditionally used for subspecies identification. Subsp. *fragile* can also be variable morphologically (**Paper I**), as also found by Armitage et al. (2017) in New Zealand. I therefore recommend that morphological separation of these subspecies should be treated with caution, and that genetic sequencing is carried out as a routine first step in work on this NNS to ensure that one is actually working with the non-native subspecies (if not available, parthenogenetic germination of gametes may be used as a substitute, but I have not evaluated this; see Trowbridge & Todd 1999a). Accurate identification is critical for studies of NNS distribution, abiotic tolerances, physiology, interactions and effects, where understanding is hampered by taxonomic confusion. Once genetic identity is established, morphological or other traits may

emerge *for that area* (e.g. reproductive timing) and be used as a proxy. More detailed examination of the morphology of these two subspecies should be done, as it may reveal characters that can be used reliably for field-use/monitoring work.

In southwestern Norway, *C. fragile* subsp. *fragile* (hereafter *C. fragile*) is distributed patchily, and occurs in the low littoral, infralittoral and sublittoral zones, with few occurrences above MLW on emergent substratum (**Paper II, III, IV**). It may also be found in tidal pools on the low- to mid-shore (**Paper I**), and down to 20 m in the sublittoral (Jorde 1966). When this and variations within other areas are considered, it appears that regional generalisations of vertical distribution (discussed in 1.5.2) become more unclear and should be reconsidered.

Figure 9 *Codium fragile* subsp. *atlanticum* in Norway (Frøya, Trøndelag). (a) A pressed specimen, ca. 25 cm long. (b, c) Photos of habitat, a low littoral tidal pool, and habit. *Photos and herbarium specimen taken by Barbro T. Haugland, figure modified from **Paper I**.*

Codium fragile has a seasonal cycle of growth and decline in southwestern Norway, but most individuals at sheltered sites retain a good portion of the thallus over winter. The first growing tips may be observed in March, demonstrating that *C. fragile* here may grow below the 10-13 °C limit proposed by Malinowski & Ramus (1973) in the NW Atlantic. It is possible that *C. fragile* has acclimatised or adapted to Norwegian temperature regimes (**Paper III**). Nevertheless, the results do not suggest that

optimum temperatures of *C. fragile* here are low, as its growth rate was higher at 15-18 °C than at 7.5 °C in experiments (but the higher temperatures were also accompanied by a longer photoperiod). Growth rate *in situ* was highest in summer, from late June to mid-September (8-10 mm per week) (**Paper III**). Fragmentation was most common from late October to February, but the alga also easily fragments in summer. My results indicate that low temperature (5 °C) is not necessary or sufficient to trigger the fragmentation process (**Paper III**).

Previous observations in Oslofjord have placed the reproductive season from July to September (Sundene 1953), which fits with my observations of mature gametangia in August (Espegrend; Fig. 10). I have also observed motile female gametes in November in samples from Tjongspollen, Bømlo, based on descriptions of Prince & Trowbridge (2004). However, this poll has some special characteristics and elevated summer temperatures (Heggøy 2001), thus may not be representative for the region. In mid-Norway, subsp. *atlanticum* had mature gametangia in November while subsp. *fragile* did not (**Paper I**). It was unfortunately not possible to characterise the gametes of these samples.

Propagule pressure and dispersal was not studied, but I found a specimen in the Oslo herbarium which demonstrates the long-distance dispersal potential of *C. fragile*. In 1930, the J. Lid expedition to Jan Mayen pressed a thallus of *C. fragile* (subspecies undetermined) which they found washed up on the island. This is at a minimum around 900 km from Norway and 1300 km from Scotland, countries where the alga might have been present at that time. This supports Gagnon et al. (2014) who suggested that buoyant *C. fragile* fragments could potentially travel hundreds of kilometres in a growth season.

► **Figure 10** Mature female gametes in a gametangium of *Codium fragile* subsp. *fragile,* in the sublittoral at Espegrend Biological Station in August 2014. The gametangium is approximately 250-300 µm long.
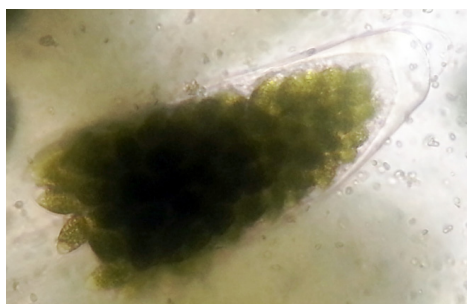
Sargassum muticum

Sargassum muticum tends to occur in the infralittoral and sublittoral zones in this region, although likely not to as deep as *C. fragile*. In my seaweed cover surveys, it was always recorded around chart datum (**Paper IV**). Olsen (2008) also reports *S. muticum* at 1-4 m depth in Oslofjord. Holdfasts are often attached to stable substratum, or small pieces of rock or bivalve shells on unstable/soft substratum.

Laterals may be around 15-20 cm in March, and depending on the suitability of the location reach 60 cm to 1 m by the end of July (**Paper V,** Study I), or longer in sounds. The alga can develop mature receptacles by late July (Study I). I have observed loss of vesicles and side branches, with epiphytic overgrowth of laterals by late July/early August at some sites (**Paper V,** Study I), while at other sites *S. muticum* may be in relatively good condition in September (Fig. 11). Measurements of growth (Study I) showed that thalli grew between 1 and 4 cm per week (mean 2.5 cm) from April to mid-May (n=50). At the site where thalli were in good condition (nutrient-enriched, low epiphytic cover) they grew between 3 and 4 cm per week (mean 3.7 cm) from mid-May to mid-June, slowing to 0 to 3 cm per week (mean 1.4 cm) in July as they became reproductive (n=10). In May and June thalli can be densely covered by filamentous brown algae (*Ectocarpus* spp. in Study I).

◀ **Figure 11** *Sargassum muticum. Top*: An example of the condition of thalli in Kuholmsundet, outside Espegrend Marine Biological Station, in early August 2015. By this point most of the short thalli had lost buoyancy, branches, and were lying flat, becoming overgrown with bryozoans. *Bottom*: Thalli in a sound in Øygarden, a few km north of the study area, in September 2014. The long thalli were still bushy with many side branches (*Photo: Pia Ve Dalen*).

2.2 Factors affecting the success of *Codium fragile*

Within the semi-exposed to sheltered range of sites examined, *C. fragile* abundance showed no relationship with wave exposure. However, its upper depth limit did, being deeper at more exposed sites (**Paper II**) (Fig. 12). The attachment of *C. fragile* may be damaged by wave action and its strength increases only slightly with size, making the alga likely to dislodge or fragment with strong waves (D'Amours & Scheibling 2007). The upper limit of *C. fragile* may therefore be deeper at semi-exposed sites because this avoids breaking waves, which create oscillating acceleration and drag forces that can dislodge thalli (Hurd 2000); alternatively, it may be because another factor at semi-exposed sites interacts with wave-force in the low- and infra-littoral to reduce success. Losses of *C. fragile* were highest in a cold winter (**Paper III**), and cold may damage thalli in the littoral (Schmidt & Scheibling 2005). It is thus possible, but untested, that a combination of emersion and low air temperatures in these zones causes damage, allowing easier dislodgement by waves. Interactions between temperature and waves have also been suggested to influence the survival of littoral *C. fragile* in the NW Atlantic (Schmidt & Scheibling 2005).

Substratum type also had an effect on *C. fragile* success, with higher abundances, an increased tendency to form a dominant zone, and deeper lower limits at sites with stony compared to bedrock substratum (Fig. 12) (**Paper II, III**). It was also related to the persistence of *C. fragile* over time: on stony substratum, *C. fragile* could remain abundant as the dominant canopy for at least 5 years, and was constantly present at most stations (16/21). In contrast, it was only consistently present at a few bedrock stations (4/25), with fluctuating presence-absence at most others. I suggest that its low abundances and limited vertical distribution on bedrock make populations more vulnerable to complete loss (**Paper III**). Stable populations on stones may provide propagules for recolonisation of these sites in a source-sink dynamic. I have observed large losses in *C. fragile* at stony sites (from high abundance to a few thalli over 1 year), but only a few times. The thalli did not appear to be replaced, with the stones mostly bare afterwards. I do not know why this occurred, but it does not seem to be a new phenomenon as Jorde (1966) reports similar occurrences.
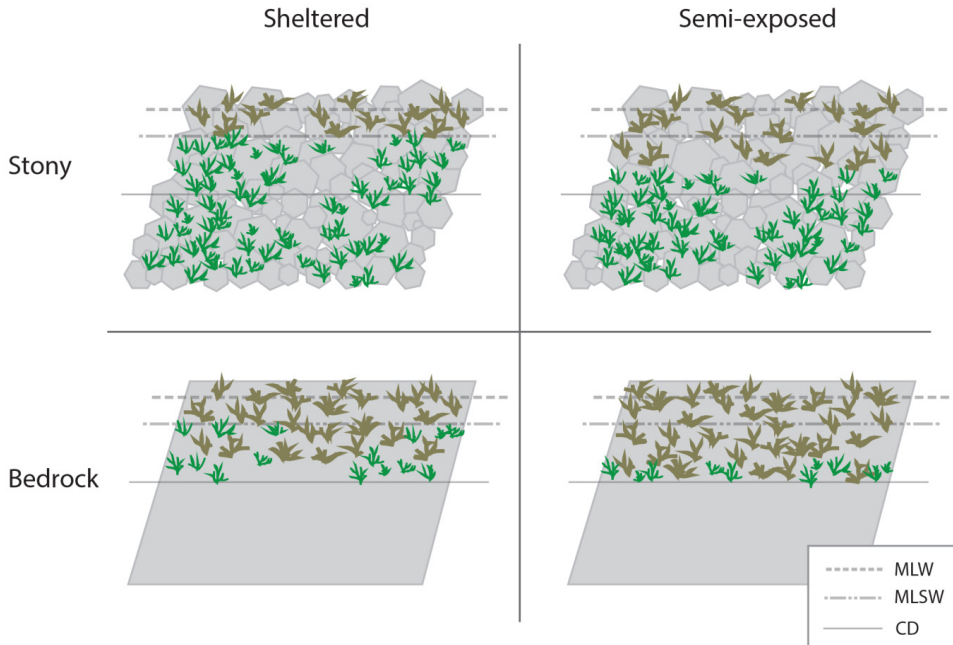
Figure 12 A schematic illustrating the typical distribution of *Codium fragile* subsp. *fragile* (green) in relation to wave-exposure, substratum, and *Fucus serratus* (brown). At stony sites *C. fragile* was often at low and high abundances, while at bedrock sites it was often only at low abundance or absent (but deviations from this were observed). The abundance of *F. serratus* was related to the abundance of *C. fragile*, but not to substratum or wave-exposure directly. Its lower limits were related to both wave-exposure and *C. fragile* abundance. The upper limits of *F. serratus* are shown as its mean upper limit across all conditions. Note that the patterns displayed are typical – e.g. *F. serratus* may also occur just below chart datum (**Paper IV**). Other species not shown. Data from **Papers II**, **III**.

I have considered several possible explanations for why the observed patterns in abundance and lower limit on the different hard substratum types occur, which would need further experiments to test. Stones may be favourable due to:

a) Propagule pressure: Gaps between stones may trap fragments of *C. fragile*, holding them in place long enough for reattachment to occur. Hydrodynamic flow patterns may also result in vortices in gaps between stones (Abelson & Denny 1997), potentially collecting small propagules (utricles, gametes).

b) Abiotic factors: Uneven surfaces disrupt water motion, dispersing wave forces and creating sheltered microhabitats. *Codium fragile* is less frequently lost from sheltered microhabitats (deeper positions in exposed tidal pools, Schmidt & Scheibling 2005; thalli surrounded by mussels on exposed breakwaters, Bulleri &

Airoldi 2005). It is therefore possible that stones can limit dislodgement of the *C. fragile* holdfast during strong waves or when it is damaged.

c) Community interactions: Small spaces between stones may act as a refuge from larger herbivores such as urchins and snails (Lubchenco 1980, Scheibling et al. 2008). Stony substratum may also be a habitat with low competition from other seaweeds. Possible reasons for this include that it is inherently disturbed, with boulders periodically turning over (Sousa 1979), or that seaweed species which grow large cannot persist on unstable substratum.

For the grazer-refuge explanation to be plausible, grazing of *C. fragile* must control its success, and grazing efficiency must be affected by substratum. I have found little evidence for the first of these statements. *Codium fragile* produces dimethylsulfonio-propionate which makes it unappealing to some herbivores (Lyons et al. 2007), and generalist grazers are not reported to control *C. fragile* in the NE Atlantic or New Zealand (Trowbridge 1995, Trowbridge & Todd 1999b, Trowbridge et al. 2016). However, this is not the case everywhere (Thomsen & McGlathery 2007), thus grazer-specific responses must be considered. Species which graze on *C. fragile* occur in Norway, such as *Elysia viridis* (Trowbridge 2002, 2004) and *Littorina littorea* (Scheibling et al. 2008), but their effects on *C. fragile* in nature have not been quantified. Urchins of *Echinus* spp. may also occur in sublittoral stony locations, but while some urchin species may eat *C. fragile*, they usually prefer kelp (Scheibling & Anthony 2001, Sumi & Scheibling 2005, Lyons & Scheibling 2008). Preferences of *Echinus* have not been tested, but they are known to eat kelp (Jones & Kain 1967) and I have not observed them grazing on *C. fragile*. Similarly, *Lacuna vincta* may be abundant on *C. fragile* but I have not seen grazing damage. The direct effect of herbivores on *C. fragile* is thus yet to be established here.

The validity of the disturbance explanation for success on stones depends on whether stony substratum is actually more disturbed, and whether *C. fragile* is facilitated by disturbance. Although unusually strong storms such as that experienced in January 2015 may cause at least small stones to move (**Paper III**), I would expect most boulders to remain relatively undisturbed at sheltered sites due to the low wave fetch.

Furthermore, in my experiment which simulated disturbance, there was no detectable benefit for *C. fragile* (**Paper IV**). However, this was only a one-off disturbance; it is possible that more frequent disturbance could have a greater effect. *Codium fragile* is probably quite a resilient species if stones do move or turn, as it may recover quickly from canopy loss by regrowth from remains between stones (**Paper IV**). A study to determine how often stones move in this area would be needed to assess this further.

It is perhaps more likely that stones would move if attached to large seaweeds. This may be why most kelps which require semi-exposed habitats tend to be associated with consolidated substratum, with the exception of *Saccharina latissima* which is associated with stones and sheltered bedrock (Kain 1979). In accordance with this, below MLSW at stony sites within this study area, *S. latissima* was much more common than *Laminaria* spp., and when it or *C. fragile* were not present, sublittoral stones were often only colonised by filamentous, turf or ephemeral seaweeds (**Papers III, IV**, pers. obs.) If *S. latissima* is the only kelp that *C. fragile* must compete for space with in the sublittoral on stones, this could contribute to its success there.

In my investigations of biotic interactions, I found indications of competition between *C. fragile* and *Fucus serratus* in the infralittoral zone. The lower depth limits of *F. serratus* were shallower and its abundance lower at sites where *C. fragile* was highly abundant (**Paper II**) (Fig. 12). As competition was not assessed directly there could be alternative explanations, but the patterns were consistent with *C. fragile* being able to limit *F. serratus* in the infralittoral at sites where it experiences good abiotic conditions. This is not a completely unexpected result, as *F. serratus* may be limited below the littoral zone by kelp (Kain & Jones 1975, Hawkins & Hartnoll 1985, **Paper V**). The results of the canopy-disturbance experiment partially support this interpretation, as clearance benefitted *F. serratus*, indicating that it was limited by other species (**Paper IV**). This suggests that *F. serratus* may recruit into gaps when seaweeds are disturbed, but seems to be a relatively poor competitor in the sublittoral (Hawkins & Harkin 1985). This may explain its success as a NNS at sublittoral sites in Nova Scotia where kelp has declined (Filbee-Dexter et al. 2016).

If *C. fragile* is limiting *F. serratus*, this is most likely by pre-emptive competition given its relatively poor interference/exploitative competition abilities (e.g. Malinowski 1974, Levin et al. 2002). For it to prevent re-establishment of native species *C. fragile* must occupy the substratum persistently or it must recruit/recover quickly. My work suggests that there is potential for this on stony substratum: *C. fragile* can attain high and lasting abundance for several years, most likely through a combination of decreased losses and higher propagule pressure (but the relative contributions of these have not been assessed) (**Paper III**). Its abundance fluctuates, sometimes greatly (Jorde 1966), but *C. fragile* can regrow quickly if fragments remain (**Paper IV**). Pre-emptive competition may therefore be the mechanism behind the observed patterns with *F. serratus*.

However, competitive relationships between *C. fragile* and *Saccharina latissima* at stony sites are unclear. In the disturbance experiment, clearance at one sheltered stony site resulted in a decrease in *C. fragile* and an increase in *S. latissima*; but the opposite at the other site (**Paper IV**). Un-investigated local abiotic or biotic conditions are probably involved in interactions between these two species. Grazing and epiphytism probably disproportionately affect *S. latissima,* as herbivores and bryozoans tend to prefer kelps to *C. fragile* (e.g. Scheibling & Anthony 2001, Levin et al. 2002, Sumi & Scheibling 2005, Lyons & Scheibling 2008). These factors may also combine, as bryozoan-encrusted *S. latissima* is a preferred food of *Echinus* spp. (Bonsdorff & Vahl 1982; Fig. 13). Furthermore, complex stony substratum creates crevices and hiding places which can reduce predation on urchins (Scheibling & Hamm 1991, Hereu et al. 2005). Thus if *Echinus* grazing of *S. latissima* is important in controlling its abundance (as it can be with kelp; Jones & Kain 1967), and *C. fragile* benefits from a reduction of this kelp, urchin grazing could contribute to the success of *C. fragile* on sublittoral stones. Further experiments are needed to investigate whether this could be the case.

In contrast to on stones, *C. fragile* shows little potential for pre-emptive competition on bedrock in the tidal zones examined. At most bedrock sites, *C. fragile* populations were unable to persist over several years, and did not become abundant. The survey

► **Figure 13** This photo from September 2015 shows juvenile *Saccharina latissima* which have successfully recruited into a sublittoral *Codium fragile* bed. With their rapid growth rate, they are overgrowing *C. fragile*. However, they are completely covered by epiphytic bryozoans (hence the white appearance), which is likely to inhibit their survival (Harris & Tyrrell 2001).

data suggests that frequent loss of thalli, rather than a lack of colonisation, may be the cause, but this needs to be investigated (**Paper III**). In the infralittoral and sublittoral at bedrock sites, *Laminaria digitata* may be common in addition to *S. latissima*. Clearance studies suggest that this species may outcompete both *F. serratus* and *S. latissima* in the sublittoral, particularly at slightly wave-exposed sites with regards to *S. latissima* (Hawkins & Harkin 1985). If *L. digitata* is a strong competitor, and *C. fragile* is less able to compete pre-emptively, this kelp may be involved in limiting *C. fragile* from the sublittoral at bedrock sites.

Disturbance of the seaweed canopy did not benefit *C. fragile*, in contrast to expectations (**Paper IV**). The alga did however recover quickly from clearance. Where recovery was very rapid this was probably related to its ability to regrow from fragments or holdfasts which remained between stones. On the other hand, propagules are required for expansion, and their settlement and growth probably takes longer. It could nonetheless occur, as shown by colonisation of previously uninhabited transects (**Paper IV**). I think that the discrepancy between my results and the expected effect of disturbance is partially due to the many possible scales, intensities, types and magnitudes of 'disturbance', and how they interact with local heterogeneity. For example, studies which have indicated positive effects of disturbance often have been done in a uniform habitat (Gagnon et al. 2014), or repeatedly cleared native algae (Scheibling & Gagnon 2006); but my disturbance was one-time event in a variable habitat (the littoral-sublittoral transition zone). The initial canopy also varied in cover and composition, thus resources were probably variable even when canopy was not cleared. Therefore, overall, the relative effect of a one-

time disturbance on *C. fragile* cover may have been much smaller than the effects of abiotic factors and resource patchiness already existing in the habitat (discussed in **Paper IV**). A similar effect has been recorded for other NNS in such environments (Morelissen et al. 2016). Given the associations of *C. fragile* with wave-exposure and substratum, and its potentially variable competitive relationships at different types of sites, an experiment with a targeted approach might be able to isolate the effect of disturbance more effectively in this area. For example, clearance of sublittoral *Laminaria* spp. on bedrock could show whether this determines the shallower lower limit of *C. fragile* there.

2.3 Factors affecting the success of *Sargassum muticum*

Biotic interactions with native algae were the main focus of study for this species. In the competition experiment, the growth of *Sargassum muticum* was similar regardless of species composition of the canopy (**Paper V**). However, this does not mean that competitive effects of *Saccharina latissima* or *Fucus serratus* on the growth of *Sargassum muticum* were absent; only that the effects were no larger than the effects of intraspecific competition. Presence of a competitive effect was indicated by the slightly higher weight gain of *Sargassum muticum* when *Saccharina latissima* was in poor condition. In contrast, *Saccharina latissima* had a negative effect on the survival of *Sargassum muticum* laterals, particularly when *F. serratus* was also present (**Paper V**). This suggests that *Saccharina latissima* canopies could negatively impact *Sargassum muticum* success by limiting the ability of laterals to survive. Although this experiment could not test how this would affect the survival of the perennial holdfast, if survival of laterals is inhibited year after year, this would be expected to have an effect on holdfast condition. It could also potentially limit reproductive output if fewer laterals made it to reproductive maturity.

The lack of effect of native species on the growth of *Sargassum muticum* was somewhat surprising, but this experiment only examined interactions between adult thalli of similar heights, and this may not be the critical stage for interactions between *S. muticum* and native seaweeds (Vaz-Pinto et al. 2014). The native species are

perennial, but *S. muticum* laterals regrow each spring. Laterals of *S. muticum* would therefore be in the understorey of native canopies for the first few weeks, significantly shaded. At this stage there would probably be greater potential for competitive inhibition of *S. muticum* growth rate by native species, and experiments examining this time period should be carried out.

The canopy-clearance experiment showed that disturbance was not sufficient to increase *S. muticum* cover (**Paper IV**). This is contrary to studies showing a positive effect of disturbance on *S. muticum* invasion (e.g. Andrew & Viejo 1998, Britton-Simmons & Abbott 2008, Bertocci et al. 2015). However, the fact that this did not occur in my study may be partially due to the disturbance type and environmental variability, as discussed for *C. fragile*. For example, Bertocci et al. (2015) found that only high intensity disturbances facilitated *S. muticum*. Furthermore, *S. muticum* was not initially common at any of my sites, thus would have had to disperse there via floating fragments. Propagule pressure was therefore probably low, which can limit the response of *S. muticum* to disturbance (Andrew & Viejo 1998, Britton-Simmons & Abbott 2008). In addition, there was high cover of turf at two of the sites which may decrease recruitment success through pre-emption of space (Britton-Simmons 2006). Therefore, the results should not be interpreted as 'disturbance is unimportant for *S. muticum* success' – rather that other factors were limiting here.

The results of my canopy-competition experiment contradict the results of Strong & Dring (2011), who found no effect of *Saccharina latissima* on *Sargassum muticum*, with intraspecific competition more important for the NNS. I believe that this discrepancy is at least partly because in my experiment *Sargassum muticum* developed poor condition, reducing its competitive effect. It underwent senescence relatively early, and probably did not reach reproductive maturity (**Paper V**). The experimental site was not completely unsuitable for *S. muticum,* as individuals occurred there naturally, and this observation is not unique to that location, as I have observed thalli present but in poor condition at other sites. In contrast, I have observed populations of large and bushy *S. muticum* maintaining much better condition at some sites, often in sounds (see Chapter 2.1). Large local variations in *S.*

muticum condition have also been observed in Ireland (Baer & Stengel 2010). This variability suggests that something is limiting *S. muticum* locally at the post-juvenile stage in summer in southwestern Norway. In order to further understand its success in this region, a) this local factor(s) should be identified, and b) temperature and competition experiments could be carried out at sites where *S. muticum* is in good condition, to limit the influence of this local factor on the results.

I propose two potential candidates for this factor, as a starting point. The first is that algal epiphytes may reduce *S. muticum* success. This was suggested by Baer & Stengel (2010, 2014) and Strong et al. (2009), who observed that the alga may be densely covered by filamentous algae of the Ectocarpales in April-June at sheltered sites in Ireland. At such sites it has lower reproduction, slower growth, and earlier senescence than at more exposed sites where epiphytic growth is lower (Baer & Stengel 2010). This epiphytic growth may increase drag forces, causing reductions in length, and decrease light levels reaching the thallus considerably (Baer & Stengel 2014), resulting in little growth and thallus loss (Strong et al. 2009). Epiphytic macroalgae may also attract herbivores, which may then graze on both seaweeds (discussed by Strong et al. 2009).

Another possibility is that *S. muticum* is nutrient-limited. The alga's pseudo-perennial lifecycle may give energetic benefits and minimise risk of being dislodged (Wernberg et al. 2000), but it also means that the alga must grow rapidly and reproduce in summer, when ambient nutrients are very low (Fig. 5). Tissue nitrogen content of *S. muticum* from populations that ended in poor condition suggests that it is severely nitrogen limited by June (**Paper V**). The alga may contain only 0.6-0.7 % nitrogen by dry weight in June-August, a reduction from 1-1.3 % in May and 2 % in March (**Paper V,** Study I). If this is the case, it might explain the apparent success of *S. muticum* in sounds. These have fast water motion which may improve uptake of what little nutrients are present (Hurd 2000), but not strong oscillating waves which can break the thallus (Viejo et al. 1995). However, it is also possible that water-motion has another positive effect on thalli, such as increased gas exchange or altered pH at the thallus surface (Lüning 1990, Hurd 2000). In addition, I have not carried out a

survey of success and environmental conditions, therefore my observation of increased success in sounds needs confirmation.

The study of *S. muticum* growth at salmon farms (Study I) did not give a conclusive answer to whether epiphytes or nutrients are important. All thalli began growing at a similar rate, despite higher internal nitrogen at the farms (Fig. 14). In May and June brown filamentous epiphytes grew abundantly on the thalli (*Ectocarpus* spp.) at all sites but Farm B, where they remained at low abundance (May) or absent (June) (Fig. 15). This occurred despite the fact that the sites were not very sheltered. At the reference sites and Farm A, the thalli were losing branches and vesicles and darkening in colour beneath the filamentous epiphytic growth by June; by mid-July, these epiphytes had disappeared but numerous other organisms covered the laterals, including juvenile mussels (*Mytilus* spp.), bryozoans, and green filamentous algae (Fig. 15). In contrast, the laterals of thalli at site B remained relatively clean, with epiphytes only occurring on the basal areas. The thalli at Farm B were the only ones which maintained good condition throughout the experiment, growing fairly long, bushy, and developing mature receptacles. Thalli at the other sites lost length and were completely overgrown before reproduction (Figs. 14 and 15).

These results support the hypothesis that filamentous brown epiphytes of the Ectocarpales can limit *S. muticum* success. Here, they were associated with poorer thallus condition and increased abundance of other epiphytes. However, it is unclear why *Ectocarpus* did not become abundant at Farm B. Proximity to a salmon farm raised the internal nitrogen levels of *S. muticum* substantially (Fig. 14), but was not sufficient to result in increased success at Farm A. Therefore, nutrients are not the only factor limiting *S. muticum*. Nevertheless, the only successful thalli were at a farm site, thus nutrient enrichment may have played a role. In general, detailed documentation of where and when *S. muticum* is successful may provide a clearer path for investigation of these factors. Identifying these limiting factors should be the next step in the study of *S. muticum* to ensure that local human impacts do not inadvertently facilitate the species.

	Ref A	Ref B	Ref C	Farm A	Farm B
Nitrogen % ±SD	0.60 ±0.19	0.61 ±0.12	0.63 ±0.14	1.38 ±0.24	1.20 ±0.09
Carbon % ± SD	27.3 ±1.45	29.3 ±3.56	28.9 ±2.56	27.3 ±0.70	33.5 ±0.26

	Ref A	Ref B	Ref C	Farm A	Farm B
Nitrogen % ±SD	1.07 ±0.07	1.03 ±0.06	1.18 ±0.04	1.78 ±0.30	1.55 ±0.11
Carbon % ± SD	29.0 ±1.24	27.2 ±3.32	25.8 ±2.13	32 ±0.61	26.5 ±2.79

	Farm B
Nitrogen % ±SD	0.84± 0.08
Carbon % ± SD	30.2± 0.51
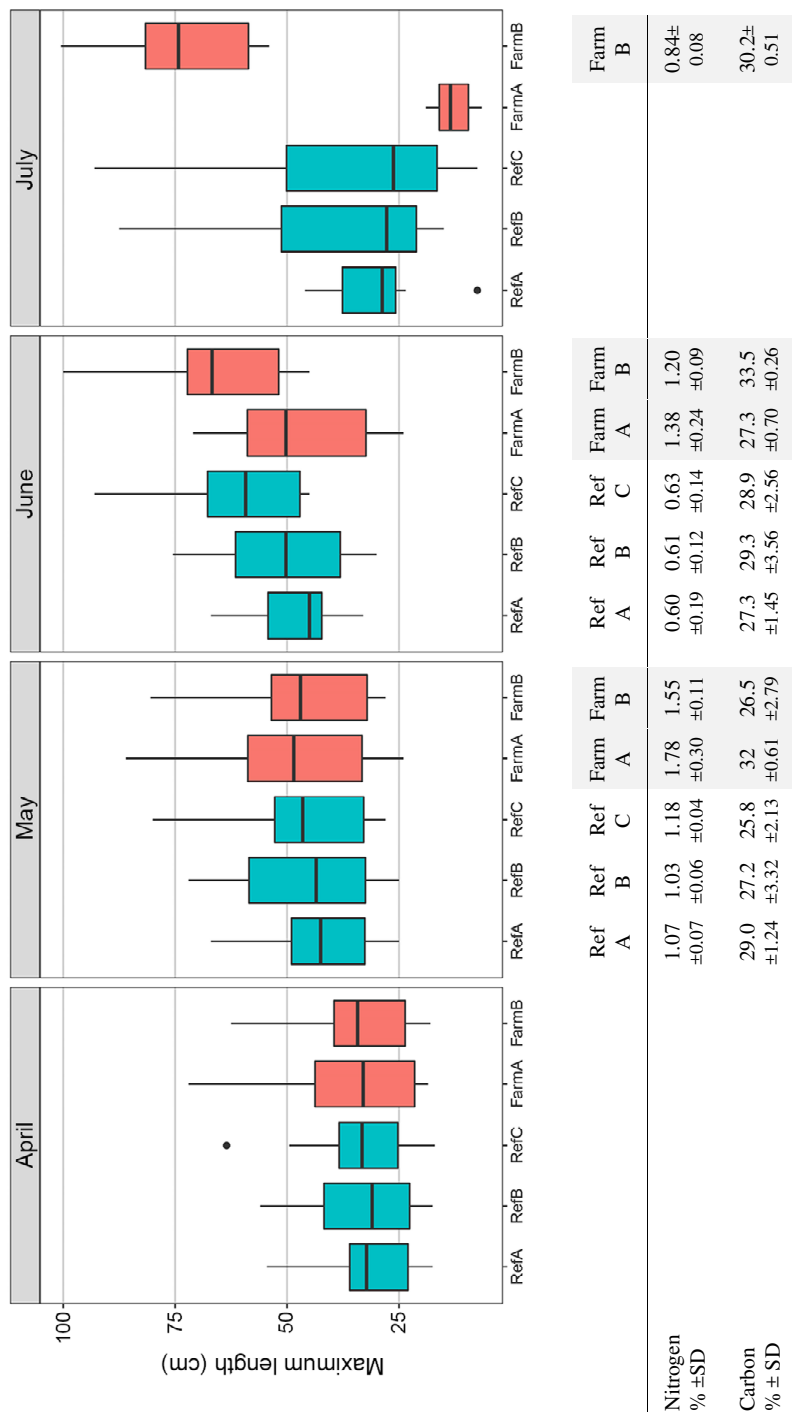
Figure 14 A boxplot of the maximum length of *Sargassum muticum* each month at each site (n = 10 per site, *blue* = reference site, *red* = farm site), with an aligned table of tissue nitrogen and carbon as a percentage of dry weight (n=3 per site, SD= Standard deviation). On collection in March, thalli had 2.04 % nitrogen and 28.9 % carbon (n=5). Boxplots: Boxes show the data between the 1ˢᵗ and 3ʳᵈ quartiles, while the whiskers show data within 1.5*the interquartile range.

Figure 15 Photos of *Sargassum muticum* thalli in Study I. *Left*: Thalli on a rope at reference site A in May, beginning to be covered by brown filamentous algae, which grew thicker in June. *Right*: A close-up of a thallus from reference site C in late July, which was extensively colonised by mussels.

2.4 Effects of environmental change on success

For *Codium fragile*, the results suggest that increased temperatures will be beneficial. However, it is increases in the lowest temperatures that are likely to have the largest direct positive impact. In laboratory experiments, growth rate of *C. fragile* approximately doubled when the temperature was raised from 5.1 to 7.4 °C, while effects of an increase from 15 to 18 °C were more unclear. Even if higher summer temperatures were to increase growth rates, the onset of fragmentation in October (when days become short) would likely prevent any increases in autumn growth. Likewise, in surveys, *C. fragile* was present at fewer sites and decreased in abundance after a cold winter compared to two mild winters, but there was no detectable effect of a hot summer. Surveys of *C. fragile* over a longer time period, containing more than one cold winter, would be needed to confirm this result. It would also be useful to have more detailed measurements of abundance, as the categories used here were relatively coarse and may have masked changes. However, increases in *C. fragile* cover after a mild winter have also been documented in the NW Atlantic (Pedersen et al. 2008). The results therefore suggest that if climate change brings milder winters, *C. fragile* would benefit in terms of faster, slightly earlier spring growth, and higher persistence (**Paper III**). If so, this could improve the ability of *C. fragile* to compete pre-emptively. It is also possible that it could extend its vertical range shallower into the littoral zone if it is currently limited by low winter air temperatures (Trowbridge 1998).

Given that previously determined optimum temperatures of *C. fragile* are relatively high (Hanisak 1979a), why did it not appear to benefit from increased summer temperatures? One reason may be that *C. fragile* is adapted or acclimatised to the current temperature regime, with current summer temperatures adequate for growth and reproduction (**Paper III**). However, the fact that I did not observe effects of temperature increase in the summer laboratory experiment, nor of a hot summer in the field, does not necessarily mean that hotter summers would have no impact on *C. fragile* success. The summer laboratory experiment was influenced by fragmentation, and only one very hot summer occurred during the 5-year survey period of field sites, thus further studies might reveal other trends. In particular, longer-term studies would probably be required to detect indirect effects of temperature, i.e. its effect on community interactions. If kelps such as *Laminaria digitata* and *Saccharina latissima* are negatively affected by higher summer temperatures, and *C. fragile* competes with these species (Levin et al. 2002, Scheibling & Gagnon 2006), *C. fragile* could indirectly benefit. *Saccharina latissima* can be strongly negatively affected by heatwaves in the upper sublittoral, and seaweeds with higher temperature tolerances can benefit from the reduction in competition (**Paper V**). However, this was not tested against *C. fragile* specifically, and a more thorough assessment of the competitive relationship between these species in this region is required. Based on the negative reaction of *Saccharina latissima* to a heatwave, and its habitat preferences, I suggest that competitive interactions with this species should be studied as a priority.

Minimum temperatures were not investigated for *Sargassum muticum,* but the effect of a hot summer was (**Paper V**). *Sargassum muticum* did not show much direct effect of higher temperature: its growth was similar between the hot and normal year, and survival of laterals was only slightly higher in the hot year (ca. 8 % increase). However, it did show a clear indirect effect of higher temperatures, due to reduced competition from *Saccharina latissima*. Its survival increased considerably (ca. 35-45 %) when *Saccharina latissima* was in poor condition due to the heat. This not only shows that temperature increases can affect competition between non-native and native seaweeds, but also that heatwaves can have a considerable effect. As *Saccharina latissima* is a widely distributed and ecologically important species in the

Atlantic, the negative effect of a heatwave documented here can be of relevance for a large area.

It was surprising that summer temperature increase did not have more direct effects on *Sargassum muticum*, as we are close to its northern limit and its optimum temperatures are high. The poor condition of *S. muticum* in both years in my experiment (**Paper V**) suggests that increases in summer temperature will be insufficient to increase *S. muticum* success at sites where it does not already maintain good condition, as local factor(s) continue to limit it. My work does not however exclude the possibility that temperature may increase success at sites where *S. muticum* already does well, or that an increase in winter/spring temperatures could improve success. In Norway generally (over and above local differences), it is also possible that certain aspects of its biology are limited by light rather than temperature. The short length of thalli in Norway compared to other locations (Engelen et al. 2015) could potentially be affected by the photoperiod: long days suppress main axis growth and promote receptacle formation (Uchida et al. 1991), and these arrive early in Norway (daylight exceeds 15 hours from the end of April to the end of August in Bergen). How this affects growth has not been established, and might indicate, as for *C. fragile*, that seasonal light cycles can limit the alga at certain times of year even if temperature does increase.

Nutrient emissions from salmon farms could clearly result in an increase in internal nitrogen in *S. muticum,* most visible in June when the farm thalli contained around double the nitrogen of thalli at the reference sites (Fig. 14). Despite this, my preliminary results do not suggest that this activity greatly increases the success of *S. muticum*. The low N values in *S. muticum* at reference sites were in line with previous measurements (**Paper V**), suggesting that the nutrient inputs from farms are not detectably absorbed at ≥ 2.8 km distance (Study I). This would make any effect on *S. muticum* quite small-scale. Additionally, even within the influence of the farms, condition of *S. muticum* was only improved at one of the two locations, showing that presence of a farm *per se* is not sufficient to improve success. However, the success of the alga in eutrophicated areas such as Limfjorden (Stæhr et al. 2000, Riisgård et

al. 2012), and the fact that the only successful thalli were at a farm site, suggests that the effects of nutrient emissions should be further investigated.

A final factor, which should perhaps be mentioned when discussing environmental change and NNS success, is the impact of other non-native species. This was not a focus of my work, but observations and knowledge of the focal species highlight two potential interactions for further study. During my surveys I often observed zones or patches of turf algae with *Bonnemaisonia hamifera* in the infralittoral zone, instead of *Fucus serratus* or kelps (**Paper III**). These patches can be maintained for at least 3 years (**Paper IV**), and it is not uncommon to find *C. fragile* in them (**Paper III**). It is possible that these patches develop where kelps and fucoids are already absent. However, *B. hamifera* has negative allelopathic effects on some macroalgae (Svensson et al. 2013) and may harbour grazers of native seaweeds (Enge et al. 2013). If these factors do not affect *C. fragile*, *B. hamifera* could potentially facilitate *C. fragile* by providing canopy-free spaces for recruitment. An investigation into this potential interaction could be useful as these seaweeds also co-occur in other regions (e.g. Harris & Tyrrell 2001, Thomsen et al. 2007). The pacific oyster, *Magallana gigas* (Thunberg, 1793) ("*Crassostrea gigas*"), is also spreading along the Norwegian coast, and may become abundant (Wrange et al. 2010, Dolmer et al. 2014). It likely acts as an ecosystem engineer, providing substratum and releasing nutrients (see discussion in Dolmer et al. 2014). *Sargassum muticum* is thought to have been introduced to Europe on the shells of oysters (Rueness 1989, Engelen et al. 2015), and accordingly, *M. gigas* has become a common substratum for *S. muticum* in Demark (Lang & Buschbaum 2010). *Codium fragile* also has the common name 'oyster thief' in the NW Atlantic due to its growth on oyster species there (Trowbridge 1998). If rugged substratum is what is behind the success of *C. fragile* on stones, spread of *M. gigas* on bedrock (or soft substratum) should be monitored to see if it facilitates *C. fragile* in those habitats.

2.5 Conclusions

The work in this thesis contributes to the understanding of abiotic and biotic factors which may influence the success of two common non-native seaweeds, and their potential reactions to temperature increase. In particular, it fills a gap by providing knowledge about these species on the Norwegian coast, a large northern habitat area in which they have been relatively understudied. The results demonstrate that even if non-native seaweeds have high temperature optima, temperature increase will not necessarily lead to increased success – the effects of temperature increase may vary by species, season, and/or local abiotic and biotic conditions. The main conclusions of this project are summarised below.

- Both *Codium fragile* subsp. *fragile* and subsp. *atlanticum* are present on the coast of Norway, but subsp. *fragile* appears to be most common in southwestern Norway. Diagnostic morphological characters can vary by location and overlap between subspecies (Paper I).

- *C. fragile* subsp. *fragile* (hereafter *C. fragile*) can be abundant at semi-exposed and sheltered sites, but its upper distribution limit tends to be shallower at the latter. Stony substratum is strongly associated with increased *C. fragile* success. On stony substratum populations can be abundant, persistent, extend into the sublittoral and form dominant canopy, while populations on bedrock tend to be ephemeral, at lower abundances, and restricted to the infralittoral (Papers II, III).

- Established *C. fragile* can persist as the dominant sublittoral canopy species for at least 5 years (Paper III).

- Distribution patterns indicate that *C. fragile* and *Fucus serratus* may compete in the infralittoral zone, and suggest that *C. fragile* may limit *F. serratus* there at stony sites (slightly more so if also sheltered) (Paper II). A one-time disturbance of the seaweed canopy did not increase *C. fragile* or *Sargassum muticum* cover, with habitat variability, and low propagule pressure for *Sargassum muticum*, probably playing a role (Paper IV). In mixed canopies of similar-sized thalli, *Sargassum muticum* growth was unaffected by canopy composition. However,

Saccharina latissima could reduce the survival of *Sargassum muticum* laterals, particularly when *F. serratus* was also present. (Paper V).

- Higher winter/spring temperatures may benefit *C. fragile* by increasing growth rates and reducing losses (Paper III), but no direct beneficial impact of hotter summers was detected for either *C. fragile* or *Sargassum muticum* (Papers III, V). However, hotter summers can alter competitive relationships between canopy seaweeds by negatively affecting heat-sensitive species (*Saccharina latissima*). When *Saccharina latissima* was suppressed, the heat-tolerant species benefitted (*Fucus serratus* and *Sargassum muticum*) (Paper V).

- Based on observations, there are likely to be local (between-site) factors which negatively affect the condition of *Sargassum muticum* in summer in this area. These local factors probably influenced the competitive relationships of *Sargassum muticum* described here, and are likely to continue limiting *Sargassum muticum* at sites where its condition is currently poor, even with temperature increase or absent *Saccharina latissima* (Paper V, Study I). Data collected were consistent with filamentous epiphytic brown algae (Ectocarpales) having a negative effect on *Sargassum muticum* growth and condition (Study I), but also suggest that it is nitrogen-limited during the summer (Paper V). Despite this, nutrient enrichment is not sufficient to improve condition or growth when epiphytic brown algae are also abundant. However, when nutrient enrichment co-occurred with few epiphytes, *Sargassum muticum* condition was good, thus both factors may be involved (Study I). This needs further investigation.

- With regards to the native canopy species examined, *Saccharina latissima* can be strongly negatively affected by heatwaves in the upper sublittoral. If in future its abundance is reduced there, the native *F. serratus* may benefit due to its positive response to disturbance, its ability to withstand heatwaves, and its competitive suppression by *Saccharina latissima* below the littoral zone (Papers IV, V).

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4. Appendicies

4.1 Study I: Methodology

On 7th March 2016, 50 thalli of *Sargassum muticum* were collected from a large, infralittoral semi-enclosed pool on Litle Tova (approximately 60.11357 N, 5.06644 E). These were then stored in a climate room at ambient seawater temperature at the University of Bergen. On 6th April, the maximum length and wet weight of each thallus was recorded, the thalli labelled, and then assigned to one of 5 groups systematically based on size (thalli arranged by length, and then assigned to a group sequentially). The thalli in each group were then attached to a rope by their holdfast at intervals of approximately 20 cm, resulting in 5 ropes, each with 10 thalli attached.

A rope was then placed at each field site on the 11th April. Two of the sites were at salmon farms, and three were at reference sites with no activity. The distance between the most distant sites was 7.5 km, with the farms spaced 2.8-3.5 km from the reference sites, and around 3.5 km from each other. The study site was slightly outside the main study area highlighted in Figure 3, but in Hordaland and in a fjord with similar abiotic conditions to the main study area. The NORWECOM.e2e model (Hjøllo et al. 2012 and references therein) and feed quantity data from the farms was used to model nutrients in the study area (Haugland et al. in preparation). This estimated the mean ambient inorganic nitrogen (ammonium, nitrite and nitrate; ± standard deviation) during the experimental period was 7.16 ±1.57 µmol l^{-1} at the farm sites, and 5.70 ±1.17 µmol l^{-1} at the control sites.

The ropes with thalli attached were held in place by attaching them at multiple points to ropes suspended horizontally between buoys. This kept the *S. muticum* holdfasts constantly submerged at a depth of approximately 0.5 m. At the farm sites, this setup was around 20-30 m from the nearest cages (Fig. A1). The sites were then visited once per month to re-measure the maximum length of the thalli, and collect tissue samples for carbon and nitrogen analysis. Visits were done on 11th May, 15th June, and 22nd July. Descriptions of epiphytes were recorded at each visit. At the final measuring point, the thalli were collected in for measurement.

Figure A1 The experimental setup at a farm site, showing *Sargassum muticum* on a rope and a fish cage in the background

Carbon (C) and nitrogen (N) content was measured in secondary branches (mid-thallus). Samples from five random thalli were taken immediately after collection in March. Once in the field, samples were taken from three thalli at each station in May and June (always the same three). In July, the condition of the most of the thalli was too poor to take samples so they were only taken from the thalli at Farm B. After collection, the samples were cleaned of visible epiphytes, dried, and ground into a fine powder. Analysis was done using a Flash 2000 elemental analyser (Thermo Fisher Scientific), in nitrogen-carbon configuration (filters, sediments, soils). The measurements were taken with the following parameters: carrier gas (helium) 130 ml min^{-1}; reference gas (helium) 100 ml min^{-1}; oxygen 250 ml min^{-1}; cycle (run time) 450 s; sampling delay 12 s; oxygen injection end 10 s (Pella & Colombo 1973).

The sites and experiment infrastructure were set up by V. Husa and B. T. Haugland (Institute of Marine Research), with B. T. Haugland also assisting with fieldwork. Seaweed CN content was measured by E. Petelenz-Kurdziel (University of Bergen). K. Sjøtun (University of Bergen) helped with collection of *S. muticum*.

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Codium fragile in Norway: subspecies identity and morphology

Abstract: The green alga *Codium fragile* consists of 10 sub-species, of which subspecies *fragile* is a well-known invasive seaweed. Morphological work carried out in the 1950s suggested that there were three subspecies along the Norwegian coast: subsp. *fragile*, subsp. *atlanticum* and subsp. *scandinavicum*. However, more recent molecular data have shown the existence of only two subspecies and that these are frequently misidentified. The aims of the present study were therefore to verify which subspecies occur in Norway using the *rpl*16-*rps*3 chloroplast marker, to ascertain their likely time of arrival and to compare their morphology to their genetic identity. DNA sequences were obtained for 60 thalli from 18 sites along the coast (57–69° N) and 10 herbarium specimens (1902–1950). The sequences indicated that both subsp. *fragile* and subsp. *atlanticum* occur at present and have been in Norway since at least 1932 and 1948, respectively. The subspecies co-occurred at one site, but in general, subsp. *atlanticum* appears to have a narrower distribution than subsp. *fragile*, both geographically and in terms of habitat. Importantly, mucron length, other utricle features, or habitat were not always sufficiently reliable to give an accurate subspecies identification, demonstrating the necessity of DNA sequencing for the identification of these subspecies.

Keywords: *Codium atlanticum*; *Codium fragile*; herbarium samples; introduced species; morphology.

Introduction

Codium fragile (Bryopsidales, Chlorophyceae) is a siphonous green alga with a NW Pacific origin (Trowbridge 1998). This taxon is presently divided into 10 subspecies (Brodie et al. 2007), one of which is a well-known introduced seaweed: *C. fragile* subsp. *fragile* (Suringar) Hariot (previously *C. fragile* subsp. *tomentosoides*; Provan et al. 2008). This subspecies has good dispersal and establishment abilities (Nyberg and Wallentinus 2005) and has spread worldwide over the last 200 years (Provan et al. 2008). In new habitats, it can have ecological and economic impacts; for example, it may compete with native kelps or fucoids (Scheibling and Gagnon 2006, Armitage et al. 2014), influence seaweed-associated fauna composition (Schmidt and Scheibling 2006, Drouin et al. 2011, Armitage and Sjøtun 2016), negatively affect commercial bivalve beds (summarised in Trowbridge 1998) and impact ecosystem services (Vilà et al. 2010). In Norway, *C. fragile* subsp. *fragile* has been classified as a high-impact non-native species due to its widespread distribution, long expected population lifetime and moderate ecological impact (Gederaas et al. 2012). In some regions, it can become locally abundant, growing in patches in the upper subtidal and infralittoral zones of sheltered and moderately wave-exposed locations, especially with boulder/cobble substratum (Armitage et al. 2014).

According to Silva (1955, 1957), *C. fragile* subsp. *atlanticum* (Cotton) Silva and subsp. *scandinavicum* Silva were already present in Norway in 1946 when subsp. *fragile* was first recorded, with the first records of subsp. *atlanticum* from 1895 and subsp. *scandinavicum* from 1929. These identifications were based on observations of utricle morphology; in particular, utricle dimensions and mucron shape and length have been used to separate the subspecies (e.g. Silva 1955, 1957, Trowbridge and Todd 1999a,b, Brodie et al. 2007). The subsp. *scandinavicum* was hypothesised to be a northern-adapted subspecies of *C. fragile* potentially originating from Siberia (Silva 1957), whereas subsp. *atlanticum* had a more southern distribution, and is listed as observed in Norway, the British Isles, France, the Netherlands, Spain and the Azores (Guiry and Guiry 2015).

However, the use of molecular methods has shown that the status and distribution of the subspecies needs re-examination. Subsp. *atlanticum* is genetically distinct from subsp. *fragile* according to a marker in the plastid genome (*rpl*16-*rps*3) and has been verified as present in the British Isles (Provan et al. 2008), but there is no genetic confirmation of its distribution in other countries to the authors' current knowledge. Furthermore,

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sequences of this marker have revealed the type material of subsp. *scandinavicum* to be the same as subsp. *fragile*, uncovering no evidence of the existence of a separate subspecies (Provan et al. 2008). More recently, a comparison of several *C. fragile* subspecies (including subsp. *fragile*, but not *atlanticum*) has suggested that subsp. *fragile* could be a separate species (Verbruggen et al. 2017).

The introduced subsp. *fragile* has often been misidentified (Provan et al. 2008). Rojo et al. (2014) found two morphological groups of *C. fragile* in NW Spain that they initially assigned to subsp. *atlanticum* and subsp. *fragile*, but the *rpl16-rps3* sequences indicated that all were subsp. *fragile*. Hubbard and Garbary (2002) and Kusakina et al. (2006) also found morphologically distinct populations of *C. fragile* in Canada, supported by genetic differences in ISSR nuclear markers (Kusakina et al. 2006), and they suggested that one might be subsp. *atlanticum*. However, later sequencing of *rpl*16-*rps3* indicated that these were two variants of subsp. *fragile* (Benton 2014). In Norway, molecular work has been done on only a few samples of *C. fragile*: Provan et al. (2008) sequenced one sample which was assumed to be subsp. *scandinavicum*, and Armitage et al. (in press) sequenced 11 samples from the Bergen area, but all of these turned out to be subsp. *fragile*.

The morphological variability of subsp. *fragile* and the common misidentification of *C. fragile* subspecies could imply two things for Norwegian records. The first is that the arrival of the non-native subsp. *fragile* in Norway could be much earlier than the first record according to Silva (1955, 1957). Observations from the 1930s describe a dramatic and obvious increase of *Codium* (notes on University Museum of Bergen herbarium specimens include: "found drifting everywhere in great quantities in the sounds in Austevoll", collected by K. Fægri in 1933; "has spread profusely in Norway in the last few years, earlier nearly unknown here" (translated), collected by H.H.H. Heiberg in 1936). This potentially reflects a rapid expansion of subsp. *fragile*, years before the first official collection (also see Fægri and Moss 1952). The second is that subsp. *atlanticum* may not actually be present in Norway, given that misidentification is common and that subsp. *atlanticum* is only confirmed from the British Isles (Provan et al. 2008).

The aims of this study were therefore (1) to sequence historical collected specimens of *C. fragile* in order to find the most likely time that subsp. *fragile* spread to Norway, (2) to check which subspecies of *C. fragile* are present in Norway, and if more than one is found, (3) to assess whether currently used micro-morphological characters are reliable for their identification. This was done by sequencing the *rps3 – rpl*16 region of the plastid genome in samples of *C. fragile* and examining the utricle morphology of the subspecies.

Materials and methods

Sampling

Fresh samples of *C. fragile* were collected along the coast of Norway between 57° and 67° N during 2014–2015 (Table 1). *C. fragile* has been recorded along the coast of Norway north to around 70° N (Stellander 1969), but is relatively rare in the southeast (Husa et al. 2013), so there were no samples from this area. Two clean branch tips around 3 cm in length from each thallus were dried in silica gel, except when the whole thallus was collected and dried as a herbarium specimen. Samples from Norwegian herbarium collections were also taken (Table 2). Herbaria contacted included the University Museum of Bergen (BG), the Botanical Museum in Oslo (O), the Norwegian University of Science and Technology Museum (TRH) and the Adger Museum of Natural History and Botanical Garden (KMN). Samples were taken of thalli which were identified as subsp. *atlanticum* in the collections (which looked in reasonable condition), along with other specimens if they were from geographical areas with poor coverage from the fresh material, or from early dates.

Sequence data

The molecular work in this study was done using methods described by Provan et al. (2008). The primers of Provan et al. (2004) were used to amplify and sequence the *rpl*16-*rps3* region of the plastid genome, which is suitable for indicating evolutionary units within the genus *Codium* (Verbruggen et al. 2007) and allows identification of subspecies of *C. fragile* using four single nucleotide polymorphisms (SNPs) (Provan et al. 2008). The UCP6 set encompasses ca. 450 bp, and three sets of primers (UCP61, 2 and 3) divide this up into smaller fragments to allow sequencing of potentially poor-quality herbarium DNA (Provan et al. 2008).

All laboratory work was carried out at the Biodiversity Laboratories (BDL, DNA section) at the University Museum of Bergen/Department of Biology (University of Bergen). DNA was extracted from a small (0.5–1 cm) section of the dried *C. fragile* using a Qiagen DNeasy

Table 1: *Codium fragile*: sequenced fresh samples from the coast of Norway (collection locations listed south to north, counties in bold).

Collection location		Date	Collector[a]	Remarks by collector	Sample code
Vest Agder					
Lillehavn	57.99302, 7.090001	Aug 2015	VH	In a harbour	70 : 1
Kilen	58.05488, 7.09849	Aug 2015	VH	On a floating dock	71 : 1
Øksnes	58.05799, 7.11044	Aug 2015	VH	On a floating dock	72 : 1
Rogaland					
Nord Talgje	59.22836, 5.78642	Jun 2014	SØ	Subtidal, fairly sheltered location	56 : 1, 56 : 3, 56 : 4, 56 : 6, 56 : 8
Hordaland					
Bømlo, Tjongspollen	59.674, 5.238	Oct-Nov 2014	KS, CSA	Thalli fertile, subtidal. Collection site is one of the few Norwegian locations where *Codium vermilara* grows (Heggøy 2000)	65 : 2, 65 : 3, 65 : 4
Austevoll, S. Huftarøy	59.99667, 5.26100	Aug 2014	SØ	Subtidal, sheltered location	63 : 1, 63 : 4
Austevoll, Rostøy	60.09187, 5.20770	Sep 2015	CSA	Thallus < 11 cm, growing on a semi-exposed vertical rock face. In a turf of *Corallina officinalis* and *Bonnemaisonia hamifera* around low water	14 : 1 (V) [KX755326]
Austevoll, Kubbholmen	60.14918, 5.16932	Mar 2014	CSA	Infralittoral/subtidal dominant patch, stony cobble substratum. Thalli ca. 20 cm, often large holdfasts (≥ 5 cm)	18 : 2, 18 : 5, 18 : 6, 18 : 7, 18 : 8
Stora Karlsøy	60.11325, 5.06491	Apr 2014	CSA	Collected from intertidal rock- pools, fairly wave exposed site. Thalli 10–15 cm, holdfasts ca. 1 cm	54 : 1, 54 : 2
Bjorøy	60.30122, 5.16673	Sep 2014	CSA	Around chart datum, patchy growth of thalli on stony cobble substratum	52 : 1 (V) [KX755329]
Lindås, Lygra	60.69869, 5.10828	Aug 2014	CSA	Floating thalli	64 : 1, 64 : 2, 64 : 3
Baløy/Nordre Sævrøyna	60.80528, 4.80860	Mar 2014	CSA	Subtidal, thalli ca. 20 cm, holdfasts often > 1 cm	53 : 9, 53 : 10, 53 : 11, 53 : 12, 53 : 13
Sogn og Fjordane					
Flora, East of Stavøya	61.54699, 5.17723	Aug 2014	MHE	Sheltered location. Some collected from a floating dock, always ca. 15 cm deep, thalli 10–15 cm. Others collected nearby, also subtidal	58 : 2, 58 : 3, 58 : 4, 58 : 5
Møre & Romsdal					
Runde, Måkeneset	62.38484, 5.60889	Jul 2014	AC	Collected from two intertidal rockpools. Small thalli (around 10 cm) arising from a basal filamentous mat	57 : 1, 57 : 4, 57 : 5, 57 : 8, 57 : 10, 57 : 12
Sør Trøndelag					
Frøya, Titran	63.66618, 8.30521	Nov 2014	OV	Grew on the shore between the quay and floating dock at Titran, in a very limited area. Samples taken within a radius of 5 m	67 : 1, **67 : 2**, 67 : 3, **67 : 4**, 67 : 5
Frøya, Hellskjæret	63.75801, 8.90768	Nov 2014	OV	–	**68 : 1, 68 : 2, 68 : 3, 68 : 4**
		June 2015	BTH	Growing in a shallow rockpool (ca. 15 × 20 m, 30–40 cm deep) with sandy/shell sand bottom on the southern tip of island. Not very abundant compared to other species, growing with *Ascophyllum nodosum*. Thalli 23–33 cm long	**68 : 8, 68 : 9, 68 : 10** (V) [KX755328]

Table 1 (continued)

Collection location		Date	Collector[a]	Remarks by collector	Sample code
Frøya, Kya	63.77967, 8.35347	June 2015	BTH	Located in shallow rockpool (ca 3 m × 1 m, 20–30 cm deep). Bedrock. *Codium* quite abundant together with coralline Rhodophyta and filamentous Chlorophyta. Thalli 15–23 cm long	**69 : 1, 69 : 2, 69 : 3** [KX755327]
Nordland					
Godøystraumen (east of Bodø)	67.24016, 14.71148	Oct 2014	KR	Sheltered site, but with current (in a channel). Thalli in a low density patch on rock, around 65 cm deep. Thalli ca. 10 cm long	66 : 1, 66 : 2, 66 : 3, 66 : 4, 66 : 5

[a]VH, Vivian Husa; SØ, Siri Ødegaard; KS, Kjersti Sjøtun; CSA, Caroline S. Armitage; MHE, Mari Heggernes Eilertsen; AC, Annelise Chapman; OV, Ola Vie; BTH, Barbro Taraldset Haugland; KR, Katrin Reiss.
The sample code is made up of "site number: sample number from that site", with sample codes in bold representing subsp. *atlanticum*, and normal font representing subsp. *fragile*. "V" indicates that thallus is stored as a herbarium specimen, and GenBank accession numbers are written in square brackets directly after the samples which they were taken from.

Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Prior to PCR amplification, the DNA extractions of the fresh samples were diluted by 50, and the herbarium samples by 10. The PCR reaction mix (25 µl total) contained 1 µl 10 µmol forward primer, 1 µl 10 µmol reverse primer, 1 µl DNA, 2 µl dNTP, 2.5 µl 10× PCR buffer, 17.35 µl ddH$_2$O, and 0.15 µl TaKaRa Taq Hot Start version (Takara Bio Inc., Otsu, Japan). The PCR was done under the following thermal settings: initial denaturation at 94°C for 5 min, 5 cycles with denaturation at 94°C for 1 min, annealing at 45°C for 90 s, and extension at 72°C for 90 s, followed by 35 cycles with 94°C for 1 min, 50°C for 90 s and 72°C for 1 min, then a final extension for 5 min at 72°C. Positive and negative controls were routinely used.

Gel electrophoresis was used to check the PCR products. A 1% agarose gel made with 1×TAE buffer (Tris base, acetic acid, EDTA) and containing GelRed (Biotium, Hayward, CA, USA) was loaded with a mix of 4 µl PCR product and 1 µl loading buffer. FastRuler DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA) and images taken with GeneSnap (SynGene, Cambridge, UK) were used to assess DNA size and quantity. PCR products were then purified in 10-µl reactions, containing 8 µl of PCR product, 0.1 µl exonuclease 1 (EXO, 10 U µL^{-1}), 1.0 µl shrimp alkaline phosphatase (SAP 10 U µL^{-1}) and 0.9 µl ddH$_2$O. Incubation at 37°C for 15 min was followed by an inactivation step at 85°C for 15 min. The BigDye (v3.1) method was used to sequence the DNA, using an Applied Biosystems 3730XL Analyzer (Thermo Fisher Scientific) at the Sequencing Facility, Molecular Biology Institute, University of Bergen (Norway).

The programme Geneious (v. 6.1, Biomatters Ltd., Auckland, New Zealand) was used to check sequences, assemble contigs and align the sequences using MUSCLE (multiple sequence comparison by log-expectation). These data were then used to ascertain subspecies identity by comparison with the sequences of Provan et al. (2008) and Benton (2014).

Morphological data

Eleven samples were examined microscopically. These came from six sites: two in Trøndelag (Hellsjæret and Titran) and four in Hordaland (Stora Karlsøy, Bjorøy, Baløy and Tjongspollen; Table 1). Dried tissue was rehydrated in seawater, and utricle morphology was examined approximately 2 cm from branch tips (the area normally used for identification and considered the most consistent; Silva 1957, Dromgoole 1975, Trowbridge 1998). Mucron length and shape was recorded for 16–20 utricles per thallus; these were selected by preparing a slide and measuring the first 20 mucrons that could be clearly seen and were not distorted. Utricles with no mucron at all were not included. Since the starting point for measuring mucron length is not clearly described in every publication, we measured it both from the inner cell wall [hereafter referred to as "length *a*", used by Kusakina et al. (2006)] and from the "shoulder" of the utricle ("length *b*"; see Supplementary Figure S1 for clarification). Utricle shape, length and width, hair scar distance to apex, and whether gametangia were present were also recorded for samples which rehydrated well, for up to 10 utricles per sample. Measurements and images

Table 2: *Codium fragile*: Norwegian herbarium samples from which sequence data could be obtained, listed by date (oldest to most recent) and with herbarium code (Herb. code).

Originally identified as	Identification by	Place of collection	Date	Collector	Herbarium code	Sample code
–	–	Møre og Romsdal, Ålesund	Aug, 1902	N. Wille	O	*OM6, OM7*
subsp. *atlanticum*	T. Levring (1937) as *f. atlanticum*	Hordaland, Herdla, Raugnøpollen	27th Jul 1932	G. Hygen, I. Jorde	BG	BM5
–	–	Vest Agder, Farsund, Vesthassel	22nd Aug 1934	O. Håversen	O	OM2
subsp. *scandinavicum > atlanticum*	P.C. Silva (1956)	Hordaland, Smekkevik, south of Brattholmen, Fjell	10th Jul 1948	E. Moss	BG	BM10
subsp. *scandinavicum* > ("slightly") *tomentosoides*	P.C. Silva (1956)	Sogn and Fjordane, Kinn (south side of inner Hovdevåg)	21st Aug 1948	E. Moss	BG	BM11
subsp. *scandinavicum*	P.C. Silva (1956)	Sogn and Fjordane, Solund, Aspø	11th Aug 1948	E. Moss	BG	**BM12**
subsp. *scandinavicum*	P.C. Silva (1956)	Nord Trøndelag, Ulsund, Ytra Vikna, Vikna	14th Jul 1949	E. Moss	BG	BM15
subsp. *scandinavicum*	P.C. Silva (1956)	Nord Trøndelag, Lovunden, Lurøy	20th Jul 1949	E. Moss	BG	BM16
subsp. *scandinavicum*	P.C. Silva (1956)	Vest Adger, Mandal	2nd Jul 1950	E. Moss	BG	BM3

Samples in bold are those genetically identified as subsp. *atlanticum*, those in normal font as subsp. *fragile*, and those in italics are neither or are doubtful (see Results).

were taken using Leica application suite software (v.4.5), Leica DFC450 camera and DM2000 LED microscope (Leica microsystems, Wetzlar, Germany).

Results

Sequence data

The sequences of the collected samples confirmed that both *C. fragile* subsp. *fragile* and subsp. *atlanticum* currently inhabit the coast of Norway. Although all of the samples from the south, southwest and northern coastlines were subsp. *fragile*, the islands around Frøya in mid-west Norway support populations of subsp. *atlanticum* (Figure 1A). This included one sampling site where both of the subspecies were growing together within a radius of around 5 m (site 67, Titran; Table 1).

Sequences of herbarium samples also showed that nearly all were subsp. *fragile,* including the earliest sequence, which was from a thallus from Hordaland in 1932 (BM5). One thallus, collected from Solund in 1948 (BM12) and originally designated as subsp. *scandinavicum* by Silva, was genotyped as subsp. *atlanticum* (Table 2, Figure 1B).

It was not possible to get sequences from a number of the herbarium samples. This included the earliest *C. fragile* in the collections (1890, Bomlø, Hordaland, B. Hansteen, O) and a floating specimen found on Jan Mayen (1930, J. Lid, O). Short sequences were obtained for the herbarium specimens OM6 and OM7, collected in Ålesund in 1902, but with unexpected results; the sequence for OM7 (136 bp) was most similar to that of *Codium vermilara*, but had four single nucleotide differences and one 3-nucleotide difference compared to the reference sequences deposited by Verbruggen et al. (2007). The OM6 sequence was short and based on only one strand, which showed double peaks at many of the sites where *C. vermilara* differs from *C. fragile*; this may be a result of contamination over the years in the herbarium.

For both subspecies, there is a single-base pair discrepancy between the sequences in the present study and the representative sequences of Provan et al. (2008) to which they were compared (GenBank accession numbers EU045560 for subsp. *fragile* and EU045559 for subsp. *atlanticum*); it has been ascertained that these are misreads in the original Provan et al. (2008) sequences (Benton 2014, personal communication J. Provan). Representative sequences from the present study have been uploaded to GenBank; subsp. *fragile* as accession number KX755326, subsp. *atlanticum* as accession number KX755327, along
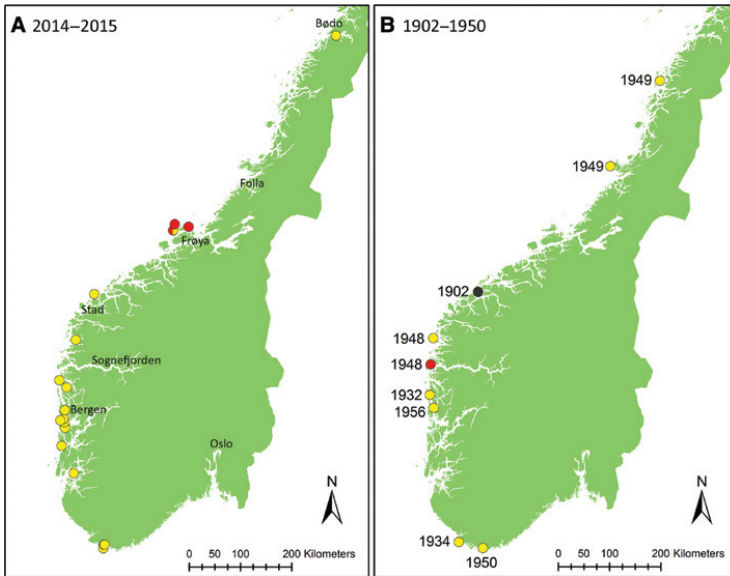
Figure 1: *Codium fragile*: Sampling sites along the coast of Norway (excluding northern Norway), showing subspecies identity at each location (subsp. *fragile* in yellow; subsp. *atlanticum* in red; uncertain or not *C. fragile* in grey) according to the *rpl*16-*rps*3 genetic marker. Map (A) shows samples from 2014 to 2015, and place names mentioned in the text; map (B) shows herbarium specimens 1902–1950 and their date of collection.

with sequences from two additional voucher specimens (whole dried thallus; Table 1).

Morphological data

In the thalli we measured, there were no consistent differences in mucron length between subsp. *fragile* and subsp. *atlanticum* (Figure 2). The seven individuals of subsp. *fragile* had a mean mucron length *a* of 14 μm (with individual thallus means ranging from 9 to 18 μm), whereas the four *atlanticum* thalli had a mean of 14 (13–18) μm. Using mucron length *b* also gave similar results for the two subspecies: 19 (15–28) μm for subsp. *fragile* and 18 (14–23) μm for subsp. *atlanticum*. With regard to mucron shape, subsp. *atlanticum* tended to have fewer pointed mucrons than subsp. *fragile*, but this character also overlapped between the two subspecies (Figures 3 and 4). In addition, both subspecies could have mucrons with fine striations (Figure 4).

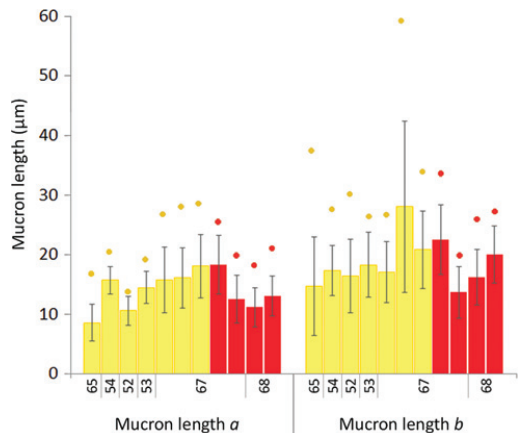Utricle widths were similar between the subspecies, at 271 μm (with individual thallus means ranging from

Figure 2: *Codium fragile* mucron lengths, as measured from the cell wall (length *a*) and the shoulder of the utricle (length *b*). Each bar represents the mean mucron length in one thallus, with standard deviation (bars) and maximum lengths (circles) shown (n = 16–20 mucrons per thallus). Site number is labelled below the bars, ordered from south to north (Table 1). Subsp. *fragile* is displayed in yellow, and subsp. *atlanticum* in red.
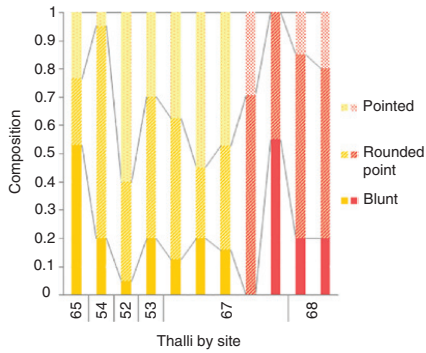
Figure 3: *Codium fragile*: The frequency of different mucron shapes observed in subsp. *fragile* (yellow) and subsp. *atlanticum* (red) thalli (n = 16–20 mucrons per thallus).
Each bar represents one thallus. Site number is labelled below the bars, ordered from south to north (Table 1). Mucron shapes are clarified in Figure 4.

241–309 μm) for subsp. *fragile* and 274 (248–299) μm for subsp. *atlanticum*. However, the utricles were slightly shorter in subsp. *fragile,* which had a mean utricle length of 634 (586–694) μm whereas *atlanticum* had a mean length of 711 (680–760) μm (Figure 5). The standard deviations of these measurements for each thallus were quite large, indicating much variation. Both subsp. *atlanticum* and *fragile* could display a constriction in the middle of the utricle (Figure 4A and B). Gametangia were present in only a few individuals; these were 351 (332–374) μm long in subsp. *atlanticum* (3 thalli, 5–10 measured per thallus), but only one mature gametangium was seen in the utricles measured for subsp. *fragile*, which was 256 μm long (see Supplementary Table S1 for full data).

There appeared to be a difference in the timing of fertility in the area where both subspecies were present. In the thalli sampled from Frøya (sites 67 and 68) in November 2014, all four subsp. *atlanticum* thalli had mature
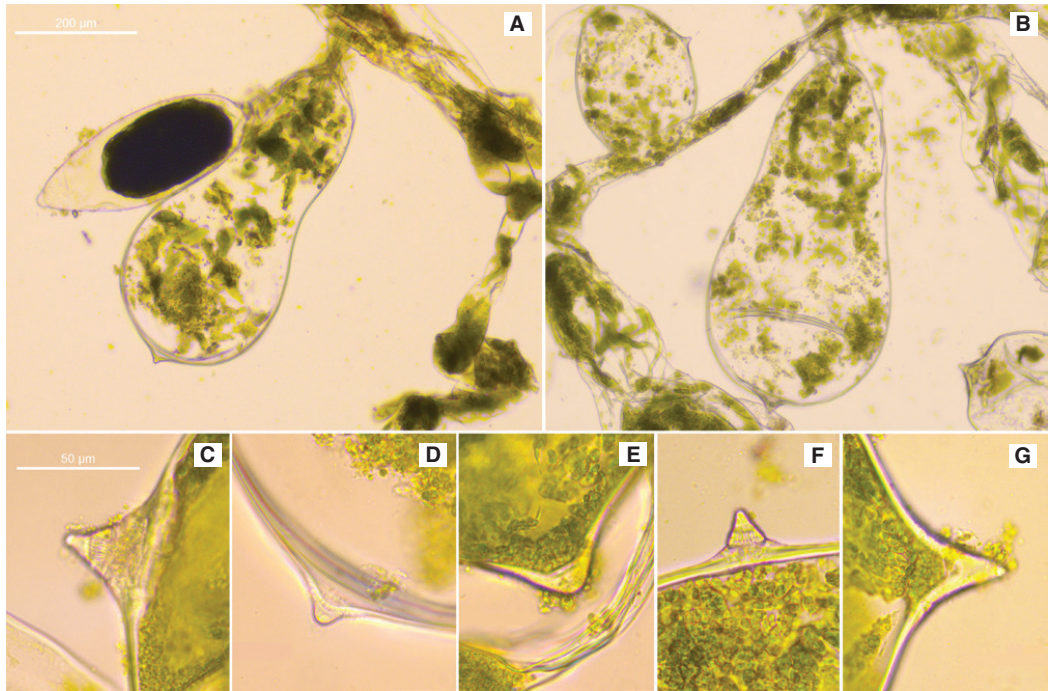

Figure 4: *Codium fragile*: Photographs of mucrons and utricles of subsp. *atlanticum* (A, C and D), and subsp. *fragile* (B, E, F and G), collected from Titran on Frøya, Norway (63.66618, 8.30521) in November 2014.
Mucrons (C) and (G) were categorised as pointed; mucrons like (E) or flatter were categorised as "blunt"; mucrons like (D) and ranging in pointedness towards (F) were categorised as "rounded points".
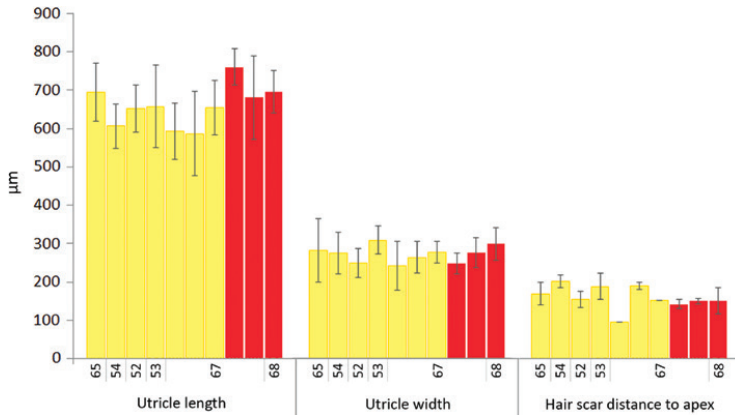
Figure 5: *Codium fragile* utricle measurements. Each bar represents the mean measurement in one thallus, with standard deviation (for utricle length and width, n = 10 utricles per thallus, except for the thallus 67 : 3, where n = 3). "Hair scar distance" refers to the distance of the hair scar from the apex of the utricle (n = 1–9 utricles per thallus). For more details of all measurements, see Supplementary Table S1. Site number is labelled below the bars, ordered from south to north (Table 1). Subsp. *fragile* is displayed in yellow, and subsp. *atlanticum* in red.

gametangia, whereas the three subsp. *fragile* thalli were infertile.

Discussion

Distribution of *Codium fragile* subspecies in Norway

By genotyping of herbarium material, the presence of *C. fragile* subsp. *fragile* was confirmed for Hordaland by 1932 and for Vest-Adger by 1934, setting back the first collection of this non-native subspecies in Norway by 14 years. However, as these two locations are approximately 300 km apart, it suggests that subsp. *fragile* had already been spreading for some time before 1932. This is supported by the fact that subsp. *fragile* had spread through the majority of its current Norwegian distribution by 1950 (approximately 1100 km of coastline), and the fact that subsp. *fragile* was already present in the Orkneys (N. Scotland) in 1891 (Provan et al. 2008). It seems likely that the dramatic increase in *Codium* along the Norwegian coast in the early 1930s (Fægri and Moss 1952) was a rapid expansion of *C. fragile* subsp. *fragile*, which was also spreading quickly in parts of Ireland during the same decade (see Trowbridge et al. 2013).

In contrast, only a few specimens of subsp. *atlanticum* were identified in the present study. This subspecies has been present in Ireland since at least 1845 (Provan

et al. 2008) and is thought to have spread northwards through Scotland on north-flowing currents (Trowbridge 1998). The distribution in Norway found in the present study is consistent with this mode of dispersal, as the current that flows northwards past the Scotland and the Shetlands tends to hit the Norwegian coast around Stad (Brattegard 2011). This current accounts for a large portion of the marine species which spread naturally to the Norwegian coast (Brattegard 2011). *C. fragile* is clearly capable of floating long-distances on currents, as shown by the drift specimen found on Jan Mayen (collected in 1930, O), which is approximately 1300 km from the Scottish mainland and 900 km from the nearest point in Norway.

Why subsp. *atlanticum* has only been found in a fairly limited region of Norway (61–64° N) compared to subsp. *fragile* has a number of possible explanations. It may be that subsp. *atlanticum* is relatively rare here, and that more samples will reveal a wider distribution. Subsp. *atlanticum* is also more uncommon and restricted in distribution than subsp. *fragile* in the British Isles, being absent from areas such as the English Channel (Brodie et al. 2007, Trowbridge and Farnham 2009). Another alternative is that subsp. *atlanticum* may require higher winter temperatures for survival than subsp. *fragile*, as seawater temperatures in winter are highest in Norway between Stad and Folla (Brattegard 2011). However, the sample from 1948 just north of Sognefjorden does not fit this pattern. Another possibility is that spread from initial colonisation sites may have been easier for the non-native subsp. *fragile* than subsp. *atlanticum*. If subsp. *atlanticum* spread to mid-Norway from the

British Isles, to expand into southern Norway, it would have to disperse against the north-flowing Norwegian coastal current and across several fjord outflows (Bakketeig et al. 2016). On the other hand, some of the earliest findings of subsp. *fragile* in the present study were from southern Norway; from here, expansion northwards along the coast could be easily achieved by drifting on the coastal current. Subsp. *fragile* can also be spread by human vectors such as boat traffic (Trowbridge 1998) and can reproduce parthenogenetically and from fragments (Churchill and Moeller 1972, Ramus 1972, Dromgoole 1975, Prince and Trowbridge 2004), meaning a only a small portion of one thallus needs to be transported to start a new population.

In the present study, the subsp. *atlanticum* samples were from rock-pools on relatively exposed islands, and the shore of a more sheltered location. No subtidal subsp. *atlanticum* was found. This fits with observations from the British Isles, where subsp. *atlanticum* has been reported to grow mainly in mid-low intertidal rock-pools (Burrows 1991) or high pools at exposed locations (Trowbridge and Todd 1999a). On the other hand, subsp. *fragile* can be found in nearly all types of habitat: the subtidal, low intertidal (around or just below mean low water), and rock-pools at both sheltered and more exposed locations. The lack of either subspecies in the mid or high intertidal is unsurprising given that occurrence on emergent substrata is relatively rare for both subspecies in areas where winter freezing occurs (Trowbridge 1998). Because subsp. *fragile* can be found in a wide range of habitats, we suggest that using tidal position or habitat for subspecies identification can be unreliable (with the possible exception of the subtidal for subsp. *fragile*, pending further investigation). The observations also suggest that subsp. *atlanticum* has a more restricted habitat than subsp. *fragile* in Norway, in addition to the more restricted geographic distribution discussed above. However, sampling at more locations is necessary to confirm this, particularly in mid-Norway and on islands at the outer edge of the coastline. Whether subsp. *atlanticum* is native or introduced is somewhat uncertain (Trowbridge 1998) but it does not appear to possess the same invasiveness as subsp. *fragile* on the Norwegian coast.

Morphology of *Codium fragile* subspecies

The results indicate that utricle morphology is not a particularly reliable character for separating *C. fragile* subsp. *fragile* and *atlanticum* in Norway. There are some trends in the utricle characters which could be related to genetic identity, but there is clearly much overlap and individual variation, making it difficult to use these characters for identification guidelines. Regarding the mucrons, most of the thalli had mean and maximum mucron lengths between 15 and 30 μm, which is intermediate between typical values used for identification of subsp. *atlanticum* and *fragile*. Only one extremely long mucron (> 40 μm) was seen, and the subsp. *fragile* mucrons were frequently shorter and blunter than expected (Silva 1957). Thus using commonly applied mucron characters for identification of these thalli would lead to misidentifications; for example that mucrons are < 15/20 μm long in subsp. *atlanticum* (Silva 1957, Burrows 1991, Brodie et al. 2007), mucrons are sharp in subsp. *fragile* (Silva 1957), and that subsp. *fragile* has fine concentric striations on the mucrons (Burrows 1991).

Utricle widths were similar between the two subspecies when from the same site, and both could display a constriction (normally only attributed to subsp. *fragile*; Silva 1957). Distance of the hair scar from the utricle apex was also quite similar: whereas the subsp. *atlanticum* samples were all within the 130–200 μm range described for subsp. *atlanticum* and below the range of 160–260 μm range for subsp. *fragile*, some of the subsp. *fragile* samples were also below 160 μm. In addition, although the subsp. *atlanticum* utricles tended to be slightly longer than the subsp. *fragile* utricles, they were generally shorter than as described for the subspecies (780–1100 μm). Their length and length/width ratio was more typical of subsp. *fragile* or subsp. "*scandinavicum*" (550–1050 and 480–850 μm, respectively; Silva 1957). There were not enough gametangia in the samples to justify a comparison in size or position between the subspecies, and all those observed were either female or, in most cases, indistinct. Determination of the mode of reproduction (parthenogenetic or sexual) has been used as a method of separating subspecies in some studies (e.g. Trowbridge and Todd 1999a) but was not investigated here as most samples were dried and/or without gametangia.

Morphological characters between the diagnostic values for each subspecies are not uncommon in Scandinavian *C. fragile*, as discussed by Silva (1957). Hybridisation has been proposed as one explanation for "intermediate" morphologies (e.g. Silva 1957, Trowbridge 1998, Kusakina et al. 2006). Theoretically, the two subspecies may be able to hybridise if a male gamete of subsp. *atlanticum* fused with a female gamete (normally parthenogenetic) of subsp. *fragile* (Trowbridge 1998). Around Frøya, the subsp. *atlanticum* thalli were fertile in November whereas the subsp. *fragile* thalli were not, suggesting reproductive separation in time – but, it is unknown whether an overlap might have occurred before sampling.

However, when considering diagnostic values, it should be taken into account that subsp. "*scandinavicum*" is likely conspecific with subsp. *fragile* (Provan et al. 2008). If so, this would mean that the described morphological differences between them may be largely due to environment (as proposed by Fægri and Moss 1952, and discussed in Silva, 1957). If this is the case, it would partially explain Silva's observation that "intergrades" are fairly common in Norway, lying between the two "morphological plateaus" of subsp. *fragile* and subsp. "*scandinavicum*" (Silva 1957), and would imply that the original morphological description of subsp. *fragile* is too narrow, in particular with regard to mucron length which can be much shorter within individuals of subsp. *scandinavicum* (described as up to 20 μm; Silva 1957). Some "intermediate" characters between subsp. *fragile* and subsp. *atlanticum* may therefore actually lie within the normal range of subsp. *fragile*, rather than being a product of hybridisation. Large morphological variability in subsp. *fragile* has also been highlighted in recent work by Armitage et al. (in press) in New Zealand.

The macro-morphology of the two subspecies was not examined here, but some observations were made. It is sometimes stated that the holdfast of subsp. *fragile* is small (usually <1 cm; Brodie et al. 2007) compared to that of subsp. *atlanticum*, but personal observations of holdfast size at sites where all sequenced samples were genetically determined as subsp. *fragile* (e.g. site 18) indicate that the holdfasts can often be much larger than 1 cm in diameter and can spread out in a mossy, undifferentiated way (Supplementary Figure S2). Differences in thallus size and number of dichotomies (e.g. Trowbridge and Todd 1999b) should also be used with caution when the samples are not from the same site. The subsp. *fragile* found in the present study could occur in patches where all were only around 10 cm long, whereas subsp. *atlanticum* could be longer than the typical 25 cm (Silva 1957). A difference that may be worth further investigation is that the subsp. *atlanticum* observed in the present study seemed to have blunter branch tips than subsp. *fragile*, which had more pointed tips (see Supplementary Figures S2 and S3), but it is unknown if this is influenced by environment.

Conclusion

Both *C. fragile* subsp. *fragile* and subsp. *atlanticum* have been growing in Norway since at least 1932 and 1948, respectively, and are still present today. The distribution of subsp. *atlanticum* is consistent with spread by currents from the British Isles, and it appears to have a more restricted distribution than subsp. *fragile*, both geographically and in habitat, but more extensive sampling is needed to confirm this. There are indications of some potential differences between the subspecies in tidal position and timing of fertility, but this also needs further investigation. The results indicate that using micro-morphological or habitat characters to identify subsp. *fragile* and subsp. *atlanticum* can easily lead to misidentifications in some locations. Because the morphological characters can overlap between the subspecies, molecular identification is recommended. Genetic identification may allow future studies of the ecology and morphology of these subspecies to reveal further and more reliable differences.

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Bionotes

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II

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Correlative evidence for competition between *Fucus serratus* and the introduced chlorophyte *Codium fragile* subsp. *fragile* on the southwest coast of Norway

Abstract: The distribution of *Codium fragile* subsp. *fragile* and the native canopy-forming alga *Fucus serratus* was recorded at 51 sites in a 20 km long, sheltered region on the southwest coast of Norway. The purpose of the study was to examine if these species are potentially competing and how their distributions are related to wave-exposure and substrate. *Codium fragile* subsp. *fragile* was patchily distributed, a pattern which appears to have been sustained over time since its introduction to this area. It was almost always observed growing below mean low water, in the low intertidal and shallow subtidal. Both substrate type and wave-exposure influenced the vertical distribution of *C. fragile* subsp. *fragile*; growth occurred higher on the shore at sheltered sites and deeper in the subtidal on stony substrate. Its vertical range of growth overlapped with that of *F. serratus* and, when *C. fragile* subsp. *fragile* was abundant, *F. serratus* tended to grow higher on the shore and at lower abundances. This suggests that *C. fragile* subsp. *fragile* is affecting *F. serratus* in this area through competition, but only in the lower portion of the fucoid's vertical range and only at sites favorable for its own growth with regard to shelter and substrate.

Keywords: *Codium fragile* subsp. *fragile*; *Fucus serratus*; invasive species; macroalgal competition.

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Introduction

Invasive species can be defined as introduced species which are economically or ecologically harmful (Boudouresque and Verlaque 2002, Nyberg and Wallentinus 2005). Invasive species are recognized as a major threat to biodiversity (Invasive Species Specialist Group 2011), causing changes in the abundance of native species (Williams and Smith 2007), predation (Blackburn et al. 2004), interspecies interactions (e.g., Bjerknes et al. 2007), habitat structure (e.g., Sousa et al. 2009), community structure and production (Vila et al. 2011), parasite and pathogen dynamics (Telfer and Bown 2012), and hybridisation (e.g., Wu et al. 2013).

There are records of around 277 introduced species of macroalgae worldwide (Williams and Smith 2007) with 97% of these being unintentionally introduced, for example, through hull fouling, ballast water and aquaculture (Hewitt et al. 2007). Introduced macroalgae tend to have a negative effect on native macroalgal abundance and assemblages, but studies show a range of effects depending on the species, processes, or area studied (reviews in Schaffelke and Hewitt 2007, Williams and Smith 2007, Thomsen et al. 2009). Thus, it is probably too early to draw conclusions about general trends (Johnson 2007), especially as only 6% of introduced species of macroalgae have been studied for ecological impacts (Williams and Smith 2007).

Codium fragile subsp. *fragile* (Suringar) Hariot (previously subsp. *tomentosoides* [van Goor] Silva; see Provan et al. 2008) is considered one of the most invasive macroalgae in Europe (Nyberg and Wallentinus 2005, Johnson 2007). It has spread from its native range in the northwest Pacific and has become established in Europe, North America, Oceania, and South Africa (Provan et al. 2008, Guiry and Guiry 2012). It was thought to have been introduced to Northern Europe sometime just prior to 1900 (Silva 1955), but specimens have recently been identified from 1845 in Ireland (Provan et al. 2008). Thus, while the official earliest record of this subspecies in Norway is 1952 (Silva 1955), it is likely to have invaded before that, and possibly as early as 1895 (Silva 1957, Norwegian Biodiversity Information Centre 2012).

In Norway, *Codium fragile* subsp. *fragile* (hereafter referred to as *C. fragile*) grows in the infralittoral and subtidal zones (Norwegian Biodiversity Information Centre 2012) and has been recorded north to 70° 00′ N, 18° 40′ E (Stellander 1969). The fucoid *Fucus serratus* Linnaeus often

forms the main canopy of the lower intertidal and infralittoral fringe zones in the northeast Atlantic (Lüning 1990). There are a number of invasive species within the Fucales (Williams and Smith 2007), and *F. serratus* itself has been unintentionally introduced to Iceland (Coyer et al. 2006) and the northwest Atlantic (Brawley et al. 2009). Due to similarities in size, branching, range, substrate requirements, winter losses, perennial nature and vertical distribution between *C. fragile* and *F. serratus* there could be potential for competition between them (Table 1). In addition, previous observations in this area suggest that *C. fragile* could have replaced *F. serratus* at some localities (Jorde 1966).

Distributions of macroalgae are influenced by competition for space, light or nutrients (Lüning 1990), but are also affected by factors such as herbivory (e.g., Norderhaug and Christie 2009), pathogens (Correa 1996), and epiphytic growth (e.g., Scheibling and Gagnon 2006). Space is often a limiting factor for algae in the low intertidal-shallow subtidal, and competition for space can involve recruiting quickly into gaps or overgrowing other algae. *Codium fragile* tends towards the former, like many other invasive macroalgae (Johnson 2007). It colonizes empty space as a result of disturbance to native algal cover, and then prevents reestablishment of the native seaweeds (see Trowbridge 1998). *Codium fragile* has been able to form large subtidal patches in this way at sites previously dominated by native kelps in the northwest Atlantic (Levin et al. 2002, Scheibling and Gagnon 2006).

Physical factors such as wave-exposure and substrate also influence macroalgal distribution (Lüning 1990, Díez et al. 2003). In more exposed areas, *F. serratus* may have lower growth rates, a shorter and later reproductive period, and consist of populations of smaller plants (Knight and Parke 1950), and for *C. fragile*, exposure reduces recruit survival (Schmidt and Scheibling 2005) and increases degree of fragmentation in winter (D'Amours and Scheibling 2007). *Fucus serratus* can tolerate moderately exposed conditions very well (Knight and Parke 1950, Johnson et al. 2012) whereas *C. fragile* grows better in areas sheltered from wave exposure (Trowbridge 1995, Bulleri and Airoldi 2005) and thus at exposed sites *F. serratus* may have an advantage.

The aim of this study is to record the distributions of *C. fragile* and *F. serratus*, and assess their potential for

Table 1 *Codium fragile* subsp. *fragile* and *Fucus serratus* traits.

	Codium fragile subsp. **fragile** (Ulvophyceae, Bryopsidales)	**Fucus serratus** (Phaeophyceae, Fucales)
Vertical range	Low intertidal, the sublittoral, and in tide pools (Burrows 1991, Trowbridge and Todd 1999, Trowbridge and Farnham 2009)	Low-intertidal, infralittoral fringe (Knight and Parke 1950, Lüning 1990).
Temperature range	Survival from at least 0°C up to 30°C (Lüning 1984). Optimum growth at 24°C, with growth above 6°C (Hanisak 1979).	Survival from at least 0°C up to 25°C, optimum growth at 15°C (Lüning 1984). Cold tolerant (Lüning 1990).
Salinity range[a]	Maximum growth at 24‰ (Yang et al. 1997). No germination below 18‰ at 6–30°C (Hanisak 1979).	Maximum growth at 20–30‰, but grows well between 10‰ and 40‰ (Bird et al. 1979).
Nutrient and light requirements	Uses all forms of nitrogen, may be able to store nutrients (Hanisak 1979, Benson et al. 1983). Nutrient enhancement may increase spread (Trowbridge and Todd 1999). Optimum growth at 88 µmol m^{-2} s^{-1} in filamentous form, but at over 200 µmol m^{-2} s^{-1} as a spongy thallus (Yang et al. 1997, Nanba et al. 2005).	Nutrient enrichment with N and P over normal levels in Norway did not affect growth (Bokn et al. 2002). Saturation for growth is reached at a minimum of 100 µmol m^{-2} s^{-1} (Bird et al. 1979).
Growth	Fast in good conditions, e.g., summer a maximum of 9.6–12 cm per month in Nova Scotia (Scheibling and Gagnon 2006). Trowbridge (1998) recorded rates of 1–2 cm per month in spring/summer native populations (Oregon). Up to 1 m long, but usually around 20 cm (Rueness 1998).	Mean of 0.49–0.85 cm per week, impacted by shelter (British Isles; Knight and Parke 1950), and latitude; 4–7 cm per year in Trøndelag (Norway; Printz, 1926, cited in Knight and Parke 1950). Around 30–60 cm long (Rueness 1998).
Structure	Mostly dichotomously branching, coenocytic (Rueness 1998).	Dichotomously branching, parenchymatous (Graham et al. 2009).
Reproduction	Parthenogenic (Feldmann 1956, Churchill and Moeller 1972, Dromgoole 1975, Benson et al. 1983) or vegetative (Mathieson 2003).	Sexual and dioecious (Graham et al. 2009).
Winter losses	Fragmentation caused by wave-action and cold temperatures (Fralick and Mathieson 1972, D'Amours and Scheibling 2007).	Breakdown of receptacle-bearing branches after reproduction (Williams 1996).

[a]Salinity reported as parts per thousand (as per the original publications) – ppt values are approximately equivalent to salinity values on the practical salinity scale.

competition on the southwestern coast of Norway. This will involve answering the following questions: (i) Do *C. fragile* and *F. serratus* grow in the same vertical zone? (ii) What are the distribution patterns of *C. fragile* and *F. serratus*, and are they associated? (iii) Are these patterns related to wave-exposure and substrate?

Materials and methods

The study region was on the southwest coast of Norway near Bergen (60° 04′ N, 005° 13′ E to 60° 16′ N, 005° 13′ E; Figure 1). This coastline is made up of islands with irregular shorelines, with seawater from the Norwegian coastal current (Sætre 2007). The macroalgal vegetation is typical of the cold temperate northeast Atlantic biogeographic region (Lüning 1990).

Observations were made at a total of 51 sites, distributed over three areas with between 13 and 22 sites in each, all relatively sheltered from wave action (Figure 1). Within each area, there were sites with a variety of wave-exposures and substrate types. Observations were made during June and August 2011. Both *Codium fragile* and *Fucus serratus* are perennial, and no large changes in their vegetation structure or cover would be expected to take place
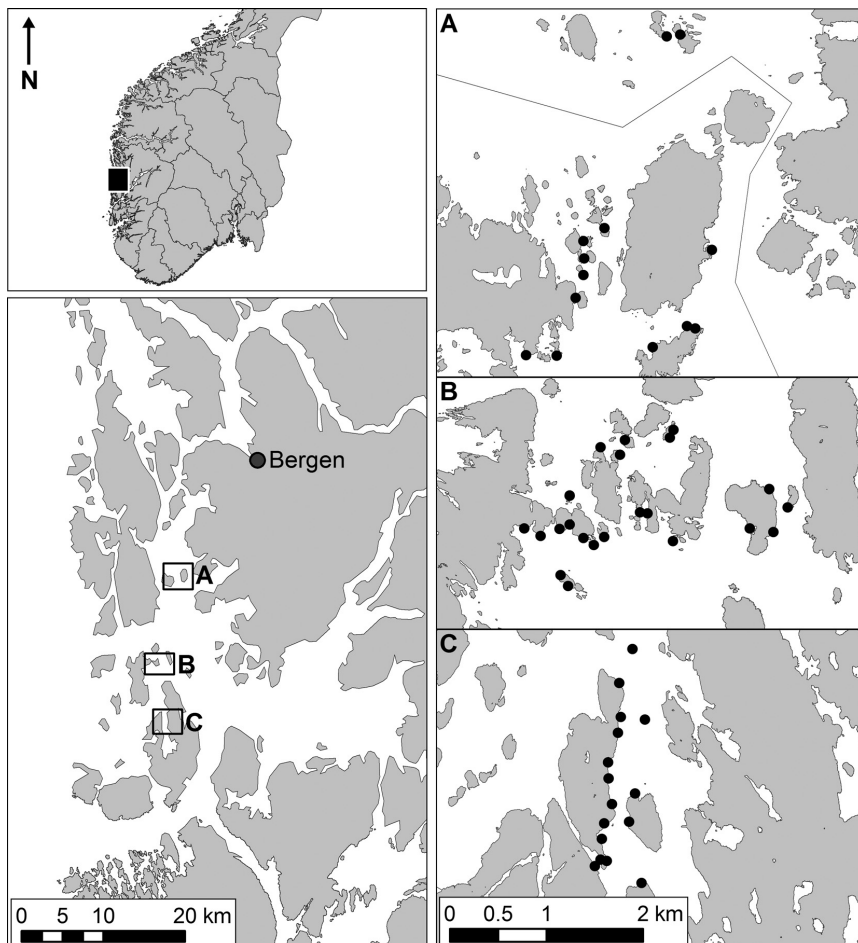

Figure 1 Location of the study. Top left is the location of the study region along the coast of Norway; bottom left shows the three areas where study sites were located; maps on the right show the locations of sites (n=51) within the three study areas.

between June and August. Possible sites on the shoreline were chosen from a distance by boat. Sites with different compositions of *F. serratus* and *C. fragile*, i.e., presence of both species or absence of one, were required in order to test possible relationship patterns between the two species. Because of this some preselected sites were discarded in order to ensure that there were sufficient sites in the different categories. In addition, only sites with hard substrate were included, and no sites facing open sea were included since *C. fragile* tends not to thrive at very exposed sites (Trowbridge 1995, Bulleri and Airoldi 2005).

Observations at each site were made around low water during spring tides using a small outboard motorboat. Observations were made along a stretch of shoreline approximately 15 m long which defined the site. The inclination was relatively high (around 20–70°) at most sites. All fieldwork was done under calm conditions and in periods with clear water and good visibility. The following field observations were made by two persons using an aquascope and a telescopic measuring rod: (i) Substrate – This was recorded as being either "rock" (solid rock face), "stones" (cobbles or boulders), or "rock, stones" (sites with a mixture). (ii) Depth of shallowest and deepest *C. fragile* and *F. serratus* to the nearest 10 cm. (iii) Distribution patterns of *C. fragile* and *F. serratus* – three main distribution patterns were observed and recorded: "zone" when the plants were in a continuous band for ≥75% of the length of the site, "patch" when the plants were growing in groups together but not as a zone, or "individuals" when the plants were mostly occurring alone among other algal species. (iv) Estimated number of *C. fragile* and *F. serratus* individuals per site, visible from the surface or using an aquascope, within categories of 0, 1–19, 20–50, or >50 plants. Because observations were made from the surface, this excluded juvenile individuals under other canopy algae. (v) Other dominant vegetation. (vi) Location (handheld GPS device, Garmin, Olathe, KS, USA).

A time record was kept for all depth measurements, which made it possible to calculate them relative to chart datum using the website of Statens Kartverk (http://www.sehavniva.no). This website provides retrospective space- and time-referenced data on observed tidal levels in Norway, based on 24 measuring stations along the coast. The heights above water were measured by placing the end of the measuring rod at water level and recording the height of the target species above the surface by sighting. Depths were measured by placing the end of the rod at the point where the target species was observed and recording the height of the water above it. In cases where it was difficult to determine the shallowest or deepest position of the two target species, more than one measurement was

made. The observed lower limit of *C. fragile* could in some cases extend to more than two metres, and in these cases, the measured lower limits are probably less accurate. When small waves were present at the site, the water level was recorded by noting the midpoint of the wave amplitudes along the measuring rod.

Measuring wave-exposure directly requires measurements of many factors over time, so a cartographic measure based on Baardseth (1970) was used. On a map (Båtsportkart, Statens Kartverk Sjøkartverket, Nordeca, scale 1:50000), straight distances from a site to the nearest land were measured at 10° intervals. These lengths were summed to give a relative exposure value for each site. To ensure that this value was reliable and not affected by chance placement of the lines, the exposure of each site was measured three times, each time randomly placing the 10° lines. From these a mean was calculated and used as the exposure value in analysis. Data of wind force and direction were not included. All the sites were relatively sheltered with small wind fetches, and in addition, there is no predominant wind direction in this area. Similar methods have been used previously in projects on littoral species (e.g., Rustad 2010). A comparison of exposure values calculated with or without wind data was done by Rustad (2010) in a comparable area, and the results showed a similar ranking of the localities.

Field measures of estimated number of plants and distribution pattern were combined to categorise sites as either low or high abundance for *C. fragile* and *F. serratus* (Table 2). The field measures of number of plants were estimates, so using them with distribution pattern improves reliability. The estimated number of plants and distribution measures were also often related, since they both reflect abundance, thus combining them gives a more integrated picture of the abundance of macroalgae at each site.

Table 2 *Codium fragile* subsp. *fragile* and *Fucus serratus:* Categorization of sites where the species were present (*C. fragile* n=36; *F. serratus* n=47) into low and high macroalgal abundance, determined by field estimates of number of individuals per site and of distribution pattern. Values are the number of sites with each combination for each species; C=*C. fragile*, F=*F. serratus*. Because the vertical distributions of *C. fragile* and *F. serratus* varied, it was possible for a site to have large numbers and zones of both species.

Field measures	1–19 plants	20–50 plants	>50 plants
Individual	Low (C=10, F=3)	Low (C=1, F=0)	–
Patch	Low (C=3, F=6)	Low (C=4, F=11)	High (C=11, F=4)
Zone	–	High (C=0, F=4)	High (C=7, F=19)

The program R (version 3.0.2, R Core team 2013) was used for statistical analyses. Significance for p-values was set at 0.05. A Kruskal-Wallis test was used to test for a relationship between wave-exposure and substrate. Substrate and wave-exposure were then used as predictor variables in binary logistic regression analysis (R package MASS; Venables and Ripley 2002) to test for a relationship between them and the abundance of *C. fragile* (the response variable – with levels "low" or "high" abundance). Sites where *C. fragile* was absent were grouped into the "low" abundance category. The model was developed using a forward selection procedure; each predictor was tested singly in a model, then models which had predictors with a significant relationship to the response variable were compared to each other by dividing the estimated standard errors by the p-values. Binary logistic regression was used firstly because the response variable was binary ("low" or "high" abundance of *C. fragile*), and secondly because this method allows the inclusion of a random factor. This random factor, "Area", was included in the model to account for possible dependency between observations, because the sites are grouped into three areas (Figure 1).

Next, the depth distribution of *C. fragile* was investigated. A linear mixed-effects model (R package nlme; Pinheiro et al. 2011) was used to see if the predictors, substrate and wave-exposure, were related to the lower limits of *C. fragile* (the response variable). Again, the random factor "area" was included. The same was then done for the upper limits of *C. fragile*. These models were developed using a standard backwards selection procedure, where Akaike's information criterion was used to compare models. One outlying observation was removed from the upper limits analysis, as it was far outside the range of the other data and was having an exaggerated effect on the analysis (45 cm below chart datum – the rest of the observations were between 5 and 50 cm above chart datum).

The same methods were then used for the two response variables, *F. serratus* abundance and lower depth limits. However, in these models, an extra predictor, *C. fragile* abundance, was included along with substrate and wave-exposure.

All of the linear models were checked for violation of assumptions, and two of the response variables required transformation. The lower limits of *F. serratus* showed heteroscedasticity which was corrected by square-root transformation. The lower limits of *C. fragile* showed problems with both heteroscedasticity and normality of errors, and a Box-Cox transformation was applied. Transformation improved both problems, but there was still some

non-normality. Therefore, a nonparametric test (Kruskal-Wallis test) was also carried out to confirm that the conclusions drawn from the linear model were reliable. The result of the binary logistic regression analysis testing for a relationship between *F. serratus* and *C. fragile* abundance (Response: *F. serratus* abundance, Predictor: *C. fragile* abundance) was also compared to a nonparametric test using the same variables (Fisher's exact test on a 2×3 contingency table of abundance) to check if the simpler analysis gave the same conclusions. Plots with confidence intervals were constructed using R package BradleyTerry2 (Turner and Firth 2012). Ninety-five percent confidence intervals (CIs) for means of upper and lower limits were calculated using ordinary nonparametric bootstrapping (R package boot; Davidson and Hinkley 1997, Canty and Ripley 2013), because much of these data were not normally distributed.

Results

The most common substrate was rock (29 sites), followed by stones and rock,stones (11 sites each). The cartographic wave-exposure of the sites ranged from 24 to 634 (mean of 225) with the majority of sites at the more sheltered end of this range; 38 out of 51 sites had values under the midpoint of the observed range (305). Substrate and exposure were not significantly associated (Kruskal-Wallis test, p=0.23). In all analyses, the standard deviation of "area" was always small compared to the standard deviation of residuals, indicating that little of the overall variation was due to differences between the three areas.

Current distribution of *Codium fragile* subsp. *fragile*

The abundance of *Codium fragile* was significantly related to substrate, but not to exposure (Table 3). There was a significantly greater chance of finding high abundance of *C. fragile* at sites where stony substrate was present (Figure 2). This was supported by our observations that at six of the 11 sites where there was both rock and stony substrate present, *C. fragile* was only observed as a patch on the stony part, remaining at low abundances on the solid rock within the same site (pers. obs.). The lower limits of *C. fragile* were also related to substrate but not to exposure, being deeper at sites with stones rather than rock (Table 3; Figure 3). On the other hand, there was a significant relationship between the upper limits of *C. fragile* and

Table 3 *Codium fragile* subsp. *fragile* and *Fucus serratus:* Statistical analyses, with response variables in bold, and predictors listed underneath. "Method" indicates statistical method used: BinaryLR, Binary logistic regression; Kruskal, Kruskal-Wallis test; LME, linear mixed-effects model; Fisher, Fisher's exact test on contingency tables. Comparisons of levels of a predictor variable (e.g. "Rock vs. Stones" within "Substrate") indicate whether there is a difference in the response variable between those levels. p-Values are rounded to 3 decimal places; those in bold are significant. Significance for multiple tests has not been corrected.

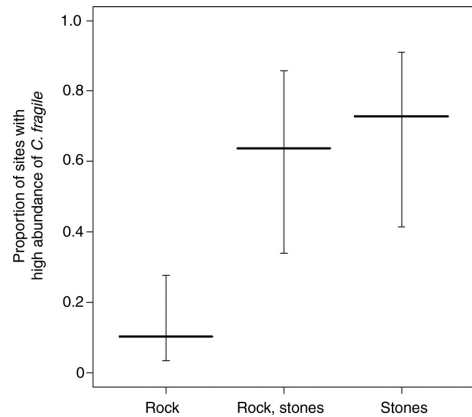| Response and predictor variables	Method	F- or t- value	p-value
C. fragile abundance (n=51)			
Substrate	BinaryLR		
Rock vs. Stones		t_{46}=-3.44	**0.002**
Rock vs. Rock, stones		t_{46}=3.02	**0.004**
Stones vs. Rock, stones		t_{46}=-0.44	0.660
Exposure	BinaryLR	t_{45}=0.08	0.936
C. fragile lower limit (n=36)			
Substrate	Kruskal		**0.009**
Substrate	LME	$F_{2,31}$=4.95	**0.010**
Rock vs. Stones		t_{31}=-2.94	**0.006**
Rock vs. Rock, stones		t_{31}=-2.53	**0.017**
Stones vs. Rock, stones		t_{31}=-0.43	0.672
Exposure	LME	$F_{1,30}$=0.491	0.489
C. fragile upper limit (n=36)			
Substrate	LME	$F_{2,29}$=0.025	0.974
Exposure	LME	$F_{1,31}$=4.17	**0.050**
F. serratus abundance (n=51)			
C. fragile abundance	Fisher		**0.031**
C. fragile abundance	BinaryLR		
Absent vs. High		t_{46}=-2.47	**0.017**
Absent vs. Low		t_{46}=-1.68	0.099
High vs. Low		t_{46}=0.98	0.333
Substrate	BinaryLR		
Rock vs. Stones		t_{44}=0.613	0.543
Rock vs. Rock, stones		t_{44}=0.668	0.102
Stones vs. Rock, stones		t_{44}=1.159	0.253
Exposure	BinaryLR	t_{45}=1.01	0.316
F. serratus lower limit (n=47)			
C. fragile abundance	LME	$F_{2,41}$=9.85	**<0.001**
Absent vs. High		t_{41}=3.12	**0.003**
Absent vs. Low		t_{41}=-1.14	0.261
High vs. Low		t_{41}=-4.71	**<0.001**
Substrate	LME	$F_{2,39}$=1.15	0.328
Exposure	LME	$F_{1,41}$=6.92	**0.009**

Figure 2 *Codium fragile* subsp. *fragile:* Proportion of sites with high abundances of *C. fragile* on different substrate types, with 95% confidence intervals ("Rock" n=29; "Rock, stones" n=11, "Stones" n=11).
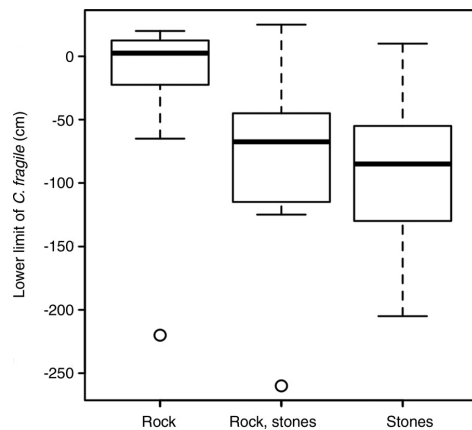

Figure 3 *Codium fragile* subsp. *fragile:* Lower limits of growth (relative to chart datum) on different substrate types ("Rock" n=16; "Rock, stones" n=10, "Stones" n=10). Upper and lower limits of boxes represent upper and lower quartiles of the data, while middle horizontal line is the median. Lines extending vertically from the boxes show the spread of remaining data, excluding the observations shown as open circles; these are values outside the upper and lower quartiles by over 1.5×the interquartile range, i.e., unusual observations.

wave-exposure but not substrate, with the upper limits being lower at more exposed sites (Table 3; Figure 4).

At rock sites, the dominant subtidal vegetation beneath the lower limit of *C. fragile* tended to be one of the native kelps *Laminaria digitata* (Hudson) J.V. Lamouroux or *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl and G. W. Saunders, with the native brown alga *Halidrys siliquosa* (Linnaeus) Lyngbye also present at 6 sites. At sites with stones ("stones" or "rock, stones"),

Saccharina latissima or *Laminaria digitata* also tended to form dominant subtidal cover next to or beneath *C. fragile* patches. However, little or no kelp was observed at two sites. At these two, along with five more of the stony sites, ephemeral filamentous brown algae were observed covering any space within patches of *C. fragile*.
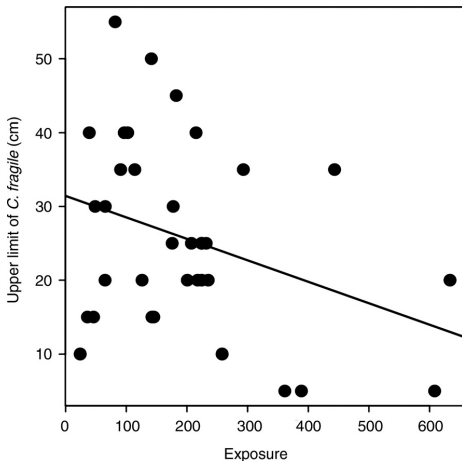
Figure 4 *Codium fragile* subsp. *fragile*: Relationship between the upper depth limit of growth (relative to chart datum) and cartographic wave-exposure (n=35).

Figure 5 *Codium fragile* subsp. *fragile* and *Fucus serratus:* Upper and lower limits of growth, relative to chart datum (n=36 for *C. fragile* and n=47 for *F. serratus*). Broken horizontal line is level of mean low water for Bergen (45 cm above chart datum). Box plots as in Figure 3.

Relationship between *Fucus serratus* and *Codium fragile* subsp. *fragile* distributions

Fucus serratus occurred most often as patches or zones and rarely as individual plants, while *Codium fragile* occurred commonly in a patchy distribution, and least often as a zone. In this study, approximately equal numbers of sites had low and high abundances of each alga (Table 2). *Codium fragile* was nearly always observed growing in the subtidal zone, extending up into the infralittoral fringe. Its mean upper limit was 24 cm (upper CI=28 cm, lower CI=17 cm) above chart datum, while its lower limits were more variable, with a mean of -54 cm (upper CI=-33 cm, lower CI=-81 cm). The mean upper limit of *F. serratus* was 56 cm (upper CI=61 cm, lower CI=53 cm), and the mean lower limit was 12 cm (upper CI=16 cm, lower CI=9 cm) (Figure 5).

There was a higher probability of *F. serratus* abundance being low at sites where the abundance of *C. fragile* was high than at sites where *C. fragile* was absent (Table 3, Figure 6). No significant relationship was found between abundance of *F. serratus* and substrate or wave-exposure. The lower limit of *F. serratus* was also significantly related to *C. fragile* abundance (Table 3, Figure 7). The lower limits of *F. serratus* were significantly higher at sites with high abundances of *C. fragile* than at sites with low or absent *C. fragile*. The mean lower limit of *F. serratus* at localities with high *C. fragile* abundance was 21 cm above chart datum (upper CI=26 cm, lower CI=15 cm), whereas the mean when *C. fragile* was absent and low was 8 cm (upper
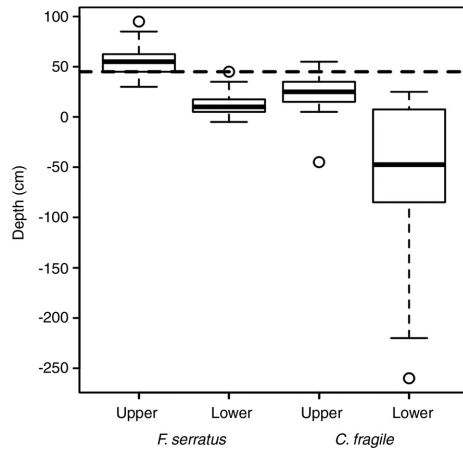
CI=10 cm, lower CI=5 cm). The lower limit of *F. serratus* was also related to wave-exposure, and was significantly higher at more sheltered sites (Table 3, Figure 7).

Discussion

Current distribution of *Codium fragile* subsp. *fragile*

Codium fragile is patchily distributed in this area, establishing in some sites as the dominant canopy but failing to establish in large numbers or to spread to others and is absent at a number of seemingly suitable sites. The same observation was made in the late 1940s in Norway (Fægri and Moss 1952), and was attributed to *C. fragile* still being in the process of dispersing into new areas. However, *C. fragile* has now been present here for so long that this explanation is inadequate. Distribution of *C. fragile* populations is also patchy in Scotland (Trowbridge and Todd 1999) and New Zealand, where presence/absence was not related to wave-exposure, bare space, herbivores or other algae and many physically suitable sites close to existing populations were not colonized (Trowbridge 1995). Thus, a patchy distribution seems to be a feature of *C. fragile* distribution in some places, rather than indicating spreading from a point of introduction.

Codium fragile was almost always observed growing below mean low water and into the subtidal zone. This
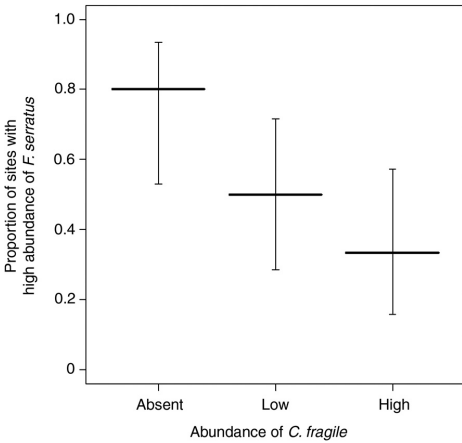
Figure 6 *Codium fragile* subsp. *fragile* and *Fucus serratus*: Proportion of sites with high abundances of *F. serratus* at different abundances of *C. fragile*, with 95% confidence intervals (*C. fragile* abundance level: "Absent" n=15, "Low" n=18, "High" n=18).
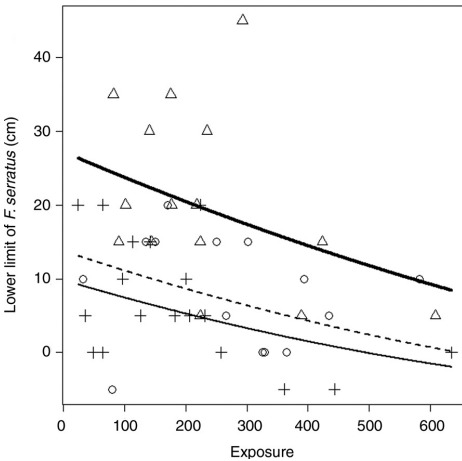

Figure 7 *Codium fragile* subsp. *fragile* and *Fucus serratus:* Lower depth limits of *F. serratus,* relative to chart datum, plotted against cartographic wave-exposure with points coded according to abundance of *C. fragile* (circle: "Absent", n=14; cross: "Low", n=18; triangle: "High", n=15). Thick line indicates relationship of *F. serratus* lower limit to wave-exposure at sites where *C. fragile* abundance was high. Thin line indicates this relationship where *C. fragile* was low, and the broken line is where *C. fragile* was absent (these two were not significantly different from each other). Regression lines are drawn based on back-transformed model parameters.

fits fairly well with other observations from the northeast Atlantic, which report *C. fragile* growing in both the lower littoral zone (in tide pools and on rock surfaces)

and the sublittoral zone (Burrows 1991, Trowbridge and Todd 1999, Trowbridge and Farnham 2009). According to Chapman (1999), the northeast and northwest Atlantic differ in that *C. fragile* tends to form small intertidal populations in the northeast Atlantic but subtidal populations in the northwest Atlantic. However the results of the present study do not support this, as *C. fragile* was observed both in the infralittoral fringe and in large subtidal patches. This is similar to other observations of its distribution in the northwest Atlantic (low intertidal and subtidal, Carlton and Scanlon 1985; subtidal, Mathieson 2003, Scheibling and Gagnon 2006), and from the native range of *C. fragile*, where it grows in the subtidal (Chavanich et al. 2006).

The distribution of *C. fragile* showed some clear patterns in relation to wave-exposure and substrate. At more exposed sites, its upper limit of growth was deeper, which may be due to damage or dislodgement by the waves near the surface. This is expected since *C. fragile* does not cope well with wave-exposure; at more exposed sites, individuals grow less "bushy" and can become fragmented with wave action (Dromgoole 1975, D'Amours and Scheibling 2007), and they tend to grow larger and more densely and to survive better in sheltered conditions (Bulleri and Airoldi 2005). If open-ocean sites had been included in the present study, one might also expect a relationship between *C. fragile* abundance and wave-exposure. However, in the relatively sheltered areas studied, *C. fragile* abundance was not related to wave-exposure. This suggests that under suitable shelter conditions other factors become important in determining abundance, one of which is likely to be substrate. The lower limits and abundance of *C. fragile* were related to substrate type, with *C. fragile* growing deeper and at higher abundances at sites with stony substrate present. *Codium fragile* can grow on many different types of substrate, such as on solid or loose rock, artificial surfaces, and in soft bottom areas through attachment to shells and eel grass rhizomes (Dromgoole 1975, Carlton and Scanlon 1985, Garbary et al. 2004, Chavanich et al. 2006), but this study indicates that stones somehow provide *C. fragile* with better conditions than an even rock surface. The causes of this were not investigated, but one potential explanation is herbivory: Scheibling et al. (2008) suggested that the impact of gastropod grazing on *C. fragile* may be substrate dependent, because settling in cracks between rocks allows macroalgal germlings to escape some herbivory (Lubchenco 1980). Alternatively, Bulleri and Airoldi (2005) found positive facilitative effects of mussel beds on *C. fragile* at exposed sites, where the rugged substrate of the mussels provided shelter for the basal parts of *C. fragile* thalli from wave

action. It may be that the large stones in this area are providing similar benefits.

Recently, in southern Norway, the kelp *Saccharina latissima* has declined by 40–80%, with losses mostly occurring at sheltered sites (Moy and Christie 2012). This is probably due to a combination of high summer temperatures, eutrophication and siltation, which have favored a shift to ephemeral algae (Moy and Christie 2012). Since availability of bare space can limit *C. fragile* recruitment (Bulleri and Airoldi 2005), this decline could be an important factor in providing opportunities for *C. fragile* in the subtidal. A reduction in native kelp may allow *C. fragile* to establish in gaps, after which cover can be maintained at physically suitable sites, i.e. sheltered and stony locations. Even in its native range *C. fragile* tends to become dominant only where the canopy-forming species have been disturbed, remaining as an understorey alga at undisturbed sites (Chavanich et al. 2006). The expansion of *C. fragile* has previously been linked to unrelated changes in the distribution of native species, e.g. decline in *Zostera marina* Linnaeus abundance due to disease (Fægri and Moss 1952). Thus, disturbance of native competitors may be an important explanatory factor in the success of *C. fragile* (Trowbridge 1998, Trowbridge and Todd 1999). Indeed, more generally, it may be that many invasive species' expansions are concurrent with unrelated declines in native competitors (Gurevitch and Padilla 2004).

In contrast to the view that there is no space monopolization by *C. fragile* in the northeast Atlantic (Schaffelke and Hewitt 2007), we observed *C. fragile* forming dominant subtidal canopy vegetation. However, it was only dominant at certain sites, and these observations were all restricted to the relatively sheltered areas studied. In addition, since this study was carried out at just one point in time, it is not known what the longevity of these patches is – whether they continue to dominate or eventually become replaced by native species again. This lack of information is an issue brought up in other work on *C. fragile* (e.g., Drouin et al. 2012) and should be addressed in future work as it is central to understanding what the impacts of *C. fragile* will be in the long-term.

Relationship between *Fucus serratus* and *Codium fragile* subsp. *fragile* distributions

Fucus serratus was very common, growing in the lower intertidal and infralittoral fringe zones as normal for this species (Knight and Parke 1950). There was an overlap between the depth ranges of *Codium fragile* and *F. serratus*

in the infralittoral fringe, with *F. serratus* then extending up into the intertidal and *C. fragile* deeper into the subtidal. As they are growing within the same vertical limits and at the same sites, this indicates there is potential for competition, but only in the infralittoral fringe.

High *C. fragile* abundance was associated with *F. serratus* growing at lower abundances, and, along with low-wave exposure, was associated with the lower limits of *F. serratus* being higher. Although the observed shift was only 13 cm, this represents a reduction of almost a third of the vertical range of *F. serratus* at sites where *C. fragile* is highly abundant. Depending on the slope of the shore, this shift can be a broad zone, detectable even using our relatively coarse measurement methods.

Wave-exposure, the upper limits and abundance of *C. fragile*, and the lower limits of *F. serratus* were all linked, but because this is a correlative study, we cannot say which is the driving force. Upper limits and abundance of *C. fragile* could be lower at exposed sites due to invasion resistance by *F. serratus*; lower limits of *F. serratus* at sheltered sites could be higher due to competition with *C. fragile*; or a third factor could be at work. An experimental approach is required to distinguish between these explanations. Having said this, *F. serratus* grows faster, larger, and has a longer reproductive period at sheltered sites (Knight and Parke 1950); thus, shelter is not expected to reduce its vertical distribution. This supports the second interpretation of relationships; favorable conditions for *C. fragile* at sheltered sites allow it to compete more effectively. This is not unlikely given that the lower limits of other fucoid species are determined by competition, while upper limits are determined by physical characteristics of the littoral zone (Lubchenco 1980, Schonbeck and Norton 1980). Recently, a decrease in effective population size and allelic richness of *F. serratus* has been shown in Norway (Coyer et al. 2008). Competition with *C. fragile* may result in additional stress for *F. serratus* in this area.

The competitive ability of *F. serratus* seems to vary depending on situation. Based on correlative work, it appears to compete well against *F. distichus* and kelp in its introduced range (Ingólfsson 2008, Johnson et al. 2012) and against *F. vesiculosus* in an area of range expansion (Arrontes 2002). In its native range, on the other hand, removal experiments have indicated that *F. vesiculosus* can be competitively superior (Jenkins et al. 1999), and that the lower limits of *F. serratus* can be determined by competition with kelp (Kain and Jones 1975). Differences in competitive ability may be due to the vertical height/ depth range studied or other unexamined factors, for example *F. serratus* may be competitively superior or inferior against the same competitor species depending

on life stage (Choi and Norton 2005a). Thus, it is difficult to assess the competitive ability of *F. serratus* against *C. fragile* without experimental work.

A number of biological or physical factors, other than direct competition, could be contributing to the distribution patterns observed. Competition is rarely only between two species, and other factors such as facilitative effects (Jenkins et al. 1999, Choi and Norton 2005b), herbivory (Lubchenco and Gaines 1981) and nutrient levels can all influence competitive relationships in macroalgae (Olson and Lubchenco 1990). *Codium fragile* may be grazed by gastropods when it is newly recruited or damaged (Scheibling et al. 2008) and can be limited when specialist herbivore densities are high (sacoglossan opisthobranchs; Trowbridge 2002), but generalist intertidal herbivores are unlikely to control *C. fragile* populations (Trowbridge 1995). Other herbivores, such as urchins (Scheibling and Anthony 2001, Sumi and Scheibling 2005), or the gastropod *Littorina obtusata* which can graze on *F. serratus* (Jenkins et al. 1999), may facilitate *C. fragile* by preferentially grazing competitor species. However, grazing by *L. obtusata* tends to occur in the midlittoral zone (Jenkins et al. 1999), and large-scale urchin grazing is restricted to northern Norway (Norderhaug and Christie 2009). In addition, we did not observe urchins or large abundances of other mesograzers during fieldwork.

Conclusion

These results suggest that localities which are sheltered and have stony substrate provide good conditions for the growth of *Codium fragile* subsp. *fragile*, allowing it to reach high abundances and grow over a greater depth range than at rock sites. In this situation *C. fragile* may supersede *Fucus serratus* in the infralittoral fringe where their ranges overlap, with the lower limit of *F. serratus* shifting

up the shore. While the present study shows correlative evidence of a competitive relationship between the two species, an experimental study is needed to prove this. However, the results are consistent with many observations of invasive macroalgae competing with and having a negative effect on native macroalgal species (Parker et al. 1999, Schaffelke and Hewitt 2007, Thomsen et al. 2009). In the northwest Atlantic, however, it has been shown that *C. fragile* is dependent upon disturbance to the closed kelp canopy for establishment within the kelp bed (Scheibling and Gagnon 2006). Whether *C. fragile* can supersede existing *F. serratus* vegetation in the infralittoral fringe or if *C. fragile* is dependent on a decrease in *F. serratus* cover in order to occupy the substratum is not known, and an experimental setup is needed to test this. In the same way, the competitive interactions between *C. fragile* and kelp species could be tested at the deeper end of the *C. fragile* depth range, given the observations of its vertical range in this study. The patchy distribution of *C. fragile* has persisted for many years along this coastline (Fægri and Moss 1952), but because *C. fragile* has a higher optimum temperature for growth than both *F. serratus* and the native kelps (Table 1, Lüning 1990), it may acquire a competitive advantage in possible future periods of higher sea surface temperatures.

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IV

V

Growth and competition in a warmer ocean: a field experiment with a non-native and two native habitat-building seaweeds

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ABSTRACT: Kelps and fucoids are important members of temperate seaweed communities, but may be negatively impacted by climate change and non-native species. We used a field experiment to investigate the effect of higher temperatures and a non-native seaweed, *Sargassum muticum*, on the kelp *Saccharina latissima* and fucoid *Fucus serratus*. All 3 are canopy-forming species which may grow together in the infralittoral and upper sublittoral zones in southwestern Norway. Artificial assemblages with different combinations of the species were placed in the shallow sublittoral, and length changes, weight changes and survival of the thalli were measured. This was done during a hot summer and again during a cool summer. The results showed that the species and their competitive interactions were affected by the different thermal conditions. *S. latissima* was the most successful species in the cool summer and had an impact on the other 2 species, but it was strongly negatively affected by the hot summer. Under these conditions, *F. serratus* became the most successful species, gaining the most weight. The effect of *Sargassum muticum* on the native species was no larger than the effect of intraspecific competition within those species. At the end of both summers *S. muticum* was in poor condition, potentially caused by low seawater nutrients resulting in low internal nitrogen.

KEY WORDS: Competition · Climate change · Non-native · Temperature effects · *Sargassum muticum* · *Saccharina latissima* · *Fucus serratus*

INTRODUCTION

Large brown macroalgae (Class Phaeophyceae) form the dominant seaweed canopy in many temperate coastal zones (Lüning 1990, Chapman 1995). Kelps and fucoids are the largest groups, and are of vital importance for coastal ecosystems: they have high primary production which enters coastal food webs (Mann 2000, Norderhaug et al. 2003, Golléty et al. 2010), structure diverse and species-rich habitats (e.g. Christie et al. 2009) and provide important ecosystem services (Smale et al. 2013).

Worryingly, there are indications that kelps and fucoids are being negatively affected by anthropogenic influences through species introductions and anthropogenic environmental changes (Smale et al. 2013, Strain et al. 2014). Non-native animals may overgrow native seaweeds (Levin et al. 2002), while non-native seaweeds may reduce the abundance of native seaweeds through competition (Scheibling & Gagnon 2006, Thomsen et al. 2016). Kelp and fucoids will also be affected by ocean warming (Brodie et al. 2014), with mean seawater temperatures predicted to rise by 2 to 3°C by 2100 in the NE Atlantic (Müller et al. 2009). Already, heat waves have been linked to declines in the abundance of kelps and fucoids, even far from range edges (e.g. Moy & Christie 2012, Smale & Wernberg 2013, Wernberg et al. 2013, Fil-

bee-Dexter et al. 2016). Interactions between stressors may also occur, resulting in a larger effect (Strain et al. 2014). For example, one non-native species may facilitate the spread of another (Levin et al. 2002), and warming and nutrient enrichment may have a synergistic negative impact on growth and survival of canopy algae (e.g. Gerard 1997, Moy & Christie 2012, Andersen et al. 2013, Strain et al. 2014). As coastal sea surface temperatures are predicted to rise (Müller et al. 2009) and the NE Atlantic is one of the most invaded areas in the world by introduced macroalgae (Thomsen et al. 2016), we can expect interactions between temperature and non-native seaweeds in this region.

Sargassum muticum (Yendo) Fensholt is a relatively well-studied, non-native seaweed which is now widespread in the NE Atlantic (Engelen et al. 2015). On the southwest coast of Norway, *S. muticum* is most common in sheltered areas or sounds where it can form locally dominant patches around or below mean low water. In these habitats, it may compete with 2 native species which also occupy the sublittoral fringe: *Saccharina latissima* (L.) Lane et al. (Laminariales) and *Fucus serratus* (L.) (Fucales), both of which are common and widely distributed in the NE Atlantic. All 3 species are canopy-forming, but have different morphologies and growth patterns (see Fig. 1), suggesting that their competitive relationships might vary depending on environmental conditions. In the sublittoral, *Sargassum muticum* competes for light by forming a floating canopy which can shade other algae below (Ambrose & Nelson 1982, Britton-Simmons 2004, Lang & Buschbaum 2010, Engelen et al. 2015), although its effect on seaweeds in the littoral zone is inconsistent (Sánchez & Fernández 2005, Sánchez et al. 2005, Harries et al. 2007, Olabarria et al. 2009, Smith 2016). Conversely, *S. muticum* can itself be limited by native species through their preemptive use of space and light (Britton-Simmons 2006, Sánchez & Fernández 2006).

As *S. muticum* on the southwestern coast of Norway is close to its European northern limit, its growth may also be limited by temperature. If so, future warming could improve conditions for *S. muticum*; firstly, by temperature ranges moving closer to its optimum (Norton 1977), and secondly, by a negative effect on cold-adapted native competitor seaweeds. *Saccharina latissima* is particularly vulnerable to increasing temperatures (Bolton & Lüning 1982, Andersen et al. 2013, Simonson et al. 2015), while fucoids such as *F. serratus* tend to be more tolerant (Lüning 1990). However, even *F. serratus* abundance has declined in parts of its southern range, and the

species is predicted to be lost from the warm temperate NE Atlantic by the year 2200 (Duarte et al. 2013, Jueterbock et al. 2013). A decline in the abundance of these species could provide opportunities for *Sargassum muticum* to establish at new sites or increase in abundance, increasing its effects on the ecosystem. The effects of *S. muticum* may vary depending on its density and the characteristics of the invaded habitat (Buschbaum et al. 2006, Lang & Buschbaum 2010, White & Shurin 2011), but in some habitats it has affected the composition of seaweed-associated fauna communities (Wernberg et al. 2004, Buschbaum et al. 2006, Harries et al. 2007, Engelen et al. 2013), influenced food webs (Salvaterra et al. 2013) and altered seasonal detritus influx (Pedersen et al. 2005).

We investigated the competitive relationships between *Saccharina latissima*, *F. serratus* and *Sargassum muticum* in a field experiment on the southwest coast of Norway, during 2 summers with widely different temperatures. The 3 large brown seaweeds represent very different morphologies and growth habits, and are predicted to vary in success depending on competitor species and environmental conditions. The aim of the study was to compare the outcome of competition between adult individuals of the 3 species, when grown together in different combinations at a fixed density. This allowed us to examine (1) the effect of a warmer than average summer on the growth and survival of the 3 species; (2) competition between the 3 species; and (3) how these 2 factors interact.

MATERIALS AND METHODS

Experimental design

The experiment was carried out on the southwest coast of Norway, which is in the cold temperate Northeast Atlantic biogeographic region (Lüning 1990). The coast is made up of many islands and fjords, resulting in a complex rocky shoreline with many areas sheltered from the open sea. Mean sea surface temperatures range from around 4.7°C in February to around 15.6°C in August (1980 to 2009 mean, 1 m depth; Institute of Marine Research 2016).

Thalli of *Sargassum muticum*, *Fucus serratus* and *Saccharina latissima* (hereafter referred to by genus alone; *Sargassum*, *Fucus*, and *Saccharina*, respectively) were collected by hand at low tide, and some *Saccharina* by triangular dredge. Collections were done within 40 km of the study site (60.26836° N, 5.21773° E). Collected thalli were stored in tanks with continuously flowing seawater or on ropes in the sea,

and were kept damp during handling. Only thalli which looked healthy were included in the experiment. The collected thalli were assembled into different combinations of the 3 species by attaching individuals to metal grids in a substitutive design. There were 7 assemblage types: (1) *Saccharina* only, (2) *Fucus* only, (3) *Sargassum* only, (4) *Saccharina* and *Fucus* mix, (5) *Saccharina* and *Sargassum* mix, (6) *Fucus* and *Sargassum* mix, and (7) *Saccharina* and *Fucus* and *Sargassum* mix. These assemblages will hereafter be referred to by an abbreviation of the species name shortened to 2 letters (*Sl* for *Saccharina*, *Fs* for *Fucus*, *Sm* for *Sargassum*; for example, *SlFs* for the *Saccharina* and *Fucus* assemblage). The single-species assemblages exposed the thalli to intraspecific competition, while the mixed-species assemblages also exposed them to different types of interspecific competition; thus the present study compared these forms of competition, rather than comparisons to no competition. Two replicates of each assemblage were made in 2014, and 3 replicates in 2015.

In all treatment types thallus density was kept constant at 50 thalli m^{-2}. In the single-species assemblages (types 1 to 3), 20 thalli of each species were used (an experimental area of 0.4 m^2); in the 2-species assemblages (types 4 to 6), 18 thalli of each species were used (0.72 m^2); and in the assemblages with all 3 (type 7), 16 thalli of each species were used (0.96 m^2). This thallus density is higher than the experimental densities of Strong & Dring (2011), but lower than Creed et al. (1998). We consider it realistic from reported densities: mature *Fucus* at 88 to 208 m^{-2} on the Isle of Mann (Creed et al. 1998); *Sargassum* at 57 m^{-2} in California (Ambrose & Nelson 1982), up to 64 m^{-2} in France (Plouguerné et al. 2006) and up to 72 m^{-2} in Ireland (Baer & Stengel 2010); *Saccharina* at 40 m^{-2} in June, close to the study site (Sjøtun 1985).

The thalli were attached so that all had approximately the same starting height (40 cm). *Fucus* thalli were trimmed at the base to avoid damaging the meristematic tips. *Sargassum* also has apical meristems, but cutting was avoided because *Sargassum* is less robust. Instead, the thalli were attached so they extended approximately 40 cm above the grid, with the basal part of the thallus in the space below the grids (which stood slightly raised from the substratum). The mean maximum length of *Sargassum* used was 56 cm, so this excess was normally only around 16 cm. *Saccharina* thalli were cut to the correct length from the distal end of the blade, as growth occurs at the stipe–blade transition (Fig. 1). *Saccharina* with stipes ≥17 cm were not included in the study; these were excluded to ensure that there was
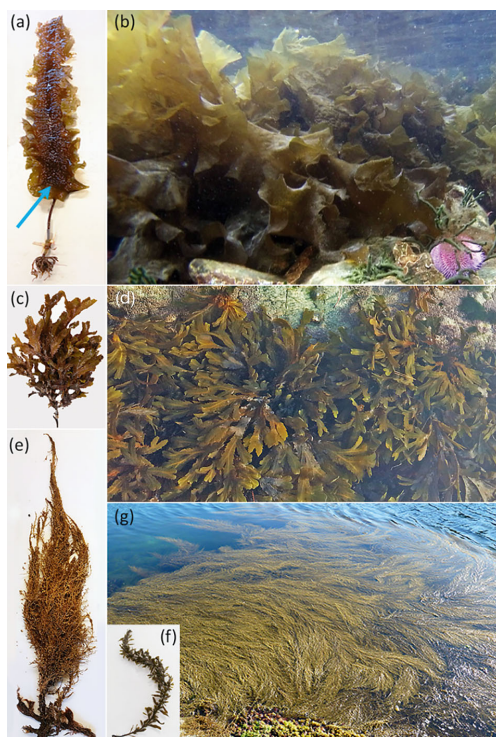

Fig. 1. Growth habits of the 3 seaweeds in southwestern Norway. (a) *Saccharina latissima* forms a broad blade, which grows from the top of the stipe (arrow). (b) The blade can increase in length quickly and lies over the substratum. (c) *Fucus serratus* has leathery, serrated, dichotomous branches with apical growth, resulting in an increasingly bushy structure over time. (d) *F. serratus* tends to spread out in a layer over the substratum. (e) *Sargassum muticum* grows rapidly from the apex and can form a long thallus (here pictured attached to a piece of rope at the base). (f) The main axis grows from early spring, becoming longer and increasingly bushy with more and longer side branches (e) by late summer. (g) Gas vesicles allow *S. muticum* to float in the water column

blade tissue left above the meristem for normal growth after trimming. In nature however, *Saccharina* stipes can often be longer than this, varying with environmental conditions and age. Standardising the starting length and thallus density meant that biomass was not standardised across the assemblage types, and varied naturally with species identity.

The length and weight of each thallus was measured before and after the experiment. For *Saccharina*, length change consists of both growth and erosion of the distal end of the blade, so these were

estimated separately by punching a hole in the blade, 10 cm above the blade–stipe transition (the location of the meristem). Tracking how far this hole moves from the stipe, in relation to the total length, is an established method for assessing kelp growth and erosion (Creed et al. 1998, Strong & Dring 2011). The age of the *Saccharina* thalli was also estimated at the end of the experiment by counting rings in a cross-section of the stipe (Parke 1948). For all species, survival was recorded for each thallus, with missing or dead individuals classed as non-surviving.

The assemblages were left in the upper subtidal from mid-May to early August, after which the thalli were collected again for re-measurement. The experimental site was outside Espegrend Marine Biological Station (University of Bergen), on the shorelines of 2 islands facing each other across a narrow channel sheltered from waves (60.26836°N, 5.21773°E). The substratum consisted of large rounded stones, colonised by turf and filamentous ephemeral algae with some natural populations of *Fucus*, *Saccharina* and *Sargassum* present. In 2014 the western island was used for both replicates, and in 2015 the eastern island was also used for the third replicate. Mean spring low water in this area is around +30 cm relative to chart datum, and the assemblages were placed around −50 cm (the deepest point on any grid was −83 cm, the shallowest point was −18 cm). Water temperature was continuously logged at 1 m depth near the site (Tinytag aquatic 2; Gemini data loggers). In 2015, seawater samples were taken to assess levels of nitrite, nitrate and phosphate (see Table 1), and were analysed according to standard methods (Parsons et al. 1992).

Seaweeds samples were also taken to measure their nitrogen and carbon content. Vegetative tissue was sampled mid-blade in *Saccharina*, and from branches in *Sargassum* and *Fucus*. In 2014, 10 *Sargassum* thalli occurring naturally near the experiment, were sampled in mid-June. In 2015, samples were taken from 7 to 10 thalli each of *Saccharina*, *Fucus* and *Sargassum* at the start of the experiment (collected from the same place as the experimental thalli; early May), 5 thalli of each species during the experiment (collected near to the experiment; late June), and 10 to 12 thalli of each species after the experiment (from the experimental thalli, evenly distributed between treatments; mid-August). Samples were analysed in a Flash 2000 elemental analyser (Thermo Fisher Scientific), in nitrogen–carbon configuration: filters, sediments, soils. The measurements were performed with the following parameters: carrier gas (helium) 130 ml min^{-1}; reference gas (helium) 100 ml min^{-1}; oxygen 250 ml min^{-1}; cycle (run time) 450 s; sampling delay 12 s; oxygen injection end 10 s (Pella & Colombo 1973).

Analysis

The number of days between start and end measurements of the thalli varied among grids due to practical limitations (from 77 to 92 d); therefore, before analysis the end length and weight measurements of each individual thallus were standardised to the shortest time (77 d), using the formula: standardised end x = start x + [(change in x / number of days) × 77], where x is length or weight. For *Saccharina* growth and erosion, the formula used was: standardised end x = [(x / number of days) × 77], where x is growth or erosion.

For each of the 3 species, analysis was carried out to ascertain how assemblage type and year influenced thallus end length, end weight and survival, as well as thallus growth and erosion in *Saccharina*. The program R (R Development Core Team 2016) was used to perform the analyses. For continuous response variables (end length, end weight, growth, erosion), linear mixed-effects models (LMMs) were used (R package 'nlme'; Pinheiro et al. 2015); for the binary response variable (survival), generalised mixed-effects models (GLMMs) were used with the binomial distribution and Laplace approximation (R package 'lme4'; Bates et al. 2015). Grid nested in replicate was included as a random effect, selected *a priori* based on experimental design. Thallus starting length or weight was also included to account for differences in starting size, and age was included for *Saccharina* as this influences growth rates (Sjøtun 1993). Nearly all *Saccharina* in the experiment were estimated to be 0 or 1 yr old; 4 thalli which were 2 yr old were excluded.

Model selection of the fixed effects was done by reducing the most complex model, including all terms and a year × treatment interaction, term-by-term, carrying out a likelihood ratio test (LRT) at each stage (Zuur et al. 2009). If the interaction was significant, the single predictors year and treatment were also retained in the model. Only the end weight of *Saccharina* showed heterogeneity of variance and was square-root transformed. If the LRT p-value was less than 0.1, the term was kept in the model, provided that there were significant differences (<0.05) in pairwise comparisons between the different levels. These pairwise differences were assessed by Wald tests on the *t*-statistic (LMMs) or *z*-statistic (GLMMs).
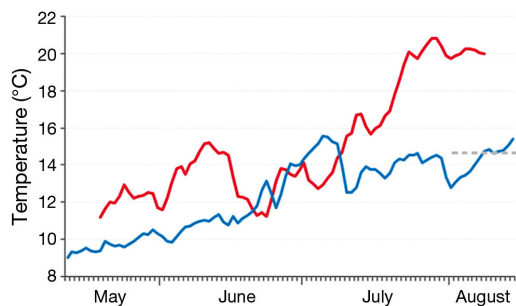
Fig. 2. Mean daily seawater temperature (1 m depth) during the experimental period in 2014 (red) and 2015 (blue). Dotted grey line: mean August sea surface temperature (1980 to 2009, 1 m depth) for this region. Note that the *y*-axis scale does not begin at 0

Table 1. Nutrients in seawater during the experimental period in 2015 (range across samples, given in µmol l^{-1}). Date format = (dd/mm/yyyy)

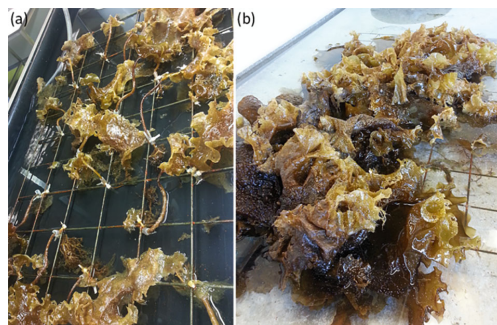| Date	No. of samples	Nitrite	Nitrate	Phosphate
27/05/2015	5	0.10−0.12	<0.4−0.5	<0.06
10/06/2015	4	0.10−0.20	<0.4	<0.06
24/06/2015	6	0.14−0.23	<0.4−0.6	<0.06−0.25
29/07/2015	3	0.19−0.24	<0.4	<0.06−0.12
11/08/2015	6	0.23−0.31	<0.4	<0.06−0.31

Fig. 3. Experimental assemblages of *Saccharina latissima* only, at the end of the experiment in (a) 2014 and (b) 2015

For the LMMs, an estimate of R^2 was obtained using the R package 'MuMIn' (Barton 2016), and plots were created using the R base package and 'ggplot2' (Wickham 2009). Least-squares means were calculated using package 'LSmeans'. Least-squares means are the predictions from the models for each assemblage type and/or year (Lenth 2016), and are provided in Figs. S1−S10 in the Supplement at www.int-res.com/articles/suppl/m573p085_supp.pdf.

RESULTS

Environmental conditions

Apart from a 3 wk period in late June/early July, daily mean temperatures were higher in the 2014 experiment (Fig. 2). The mean difference in monthly averages was 3.2°C, with overall mean temperatures of 15.1°C in 2014 versus 12.4°C in 2015. The temperature difference was most pronounced during the last part of the experiment, when in 2014 there were 17 d where mean daily seawater temperatures were at or above 19.4°C (max. recorded temperature: 21.5°C), while in 2015, the temperature never exceeded 16.2°C. The large difference in temperatures means that hereafter 2014 is referred to as the hot year, and 2015 as the cool year.

Data from the Meteorological Institute of Norway (Meteorologisk Institutt 2016) showed that cloud cover was lower in 2014 than 2015. In May, June, July and August, the total number of overcast days (defined as the number of oktas [eighths of the sky covered in cloud] at 3 measuring times summing to ≥20) was 34 in 2014, versus 57 in 2015. Mean cloud cover was similar, with both years having intermediate levels (4.3 oktas in 2014 and 5.7 oktas in 2015). Seawater nutrients were low throughout summer 2015, with no recordings of nitrate, nitrite or phosphate >0.6 µmol l^{-1} (Table 1).

Saccharina latissima

There was a large difference in the condition of *Saccharina* between the hot and cool years. In the cool year, *Saccharina* had large positive length and weight changes, higher growth and low erosion, whereas in the hot year, weight loss and length reduction frequently occurred (Figs. 3−5). Significant interactions between assemblage type and year were present for both end length and end weight (Table 2). In the cool year, the length and weight gain of *Saccharina* was fairly similar in all assemblage types. However in the hot year, the length and weight change of *Saccharina* was lower when in combination with *Fucus* (*SlFs*) than in some or all of the other assemblage types, with negative changes on average (Fig. 4, Figs. S1 & S2 in the Supplement at www.int-
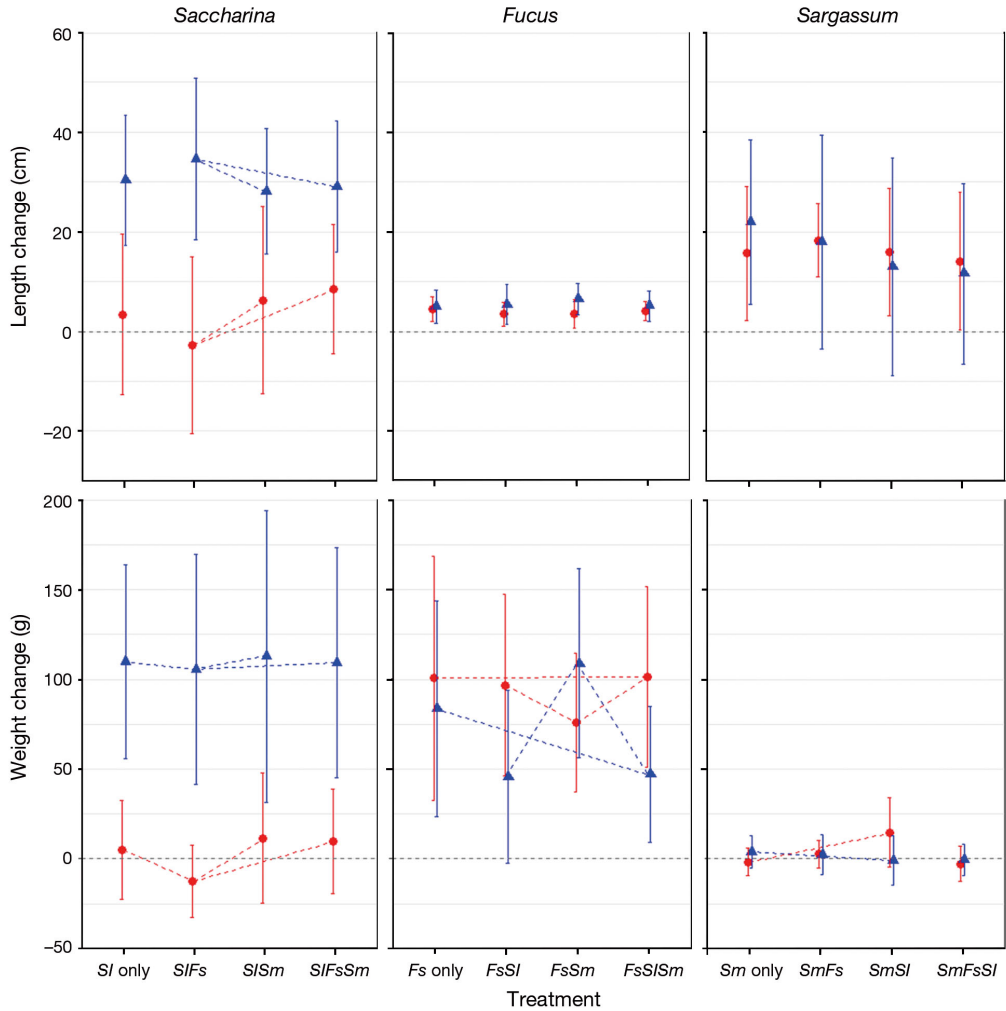
Fig. 4. Mean length change (top row) and weight change (bottom row) of *Saccharina latissima* (*Sl*; n = 133 in 2014, 177 in 2015, left column), *Fucus serratus* (*Fs*; n = 141 in 2014, 196 in 2015, middle column), and *Sargassum muticum* (*Sm*; n = 110 in 2014, 112 in 2015, right column) during the experiment in different treatments and years (red circles 2014; blue triangles 2015). Note that the order of species in the mixed-species assemblages is not important, and is only arranged so that the species of interest is first (i.e. *SlFs* is the same as *FsSl*). Significant interactions (Table 2) are shown as dotted lines between treatment types. Error bars: SD. Length change of *Saccharina* is the result of both growth and erosion of the blade, which are examined separately in Fig. 5

res.com/articles/suppl/m573p085_supp.pdf). This is likely connected to patterns in growth and erosion. *Saccharina* growth was always significantly higher in the assemblage with *Fucus* (*SlFs*) than the other treatments (Table 2, Figs. 5 & S3). However, the effect of treatment on erosion varied by year (Table 2). In the hot year, erosion increased more in the assem-

blage with *Fucus* (*SlFs*) than the other treatments (especially compared to the assemblage with *Sargassum*; *SlSm*); however, in the cool year, erosion was low in all assemblage types (Table 2, Figs. 5 & S4). Starting length and weight of *Saccharina* were positively related to end length and weight respectively, but thallus age was unrelated to both (Table 2).
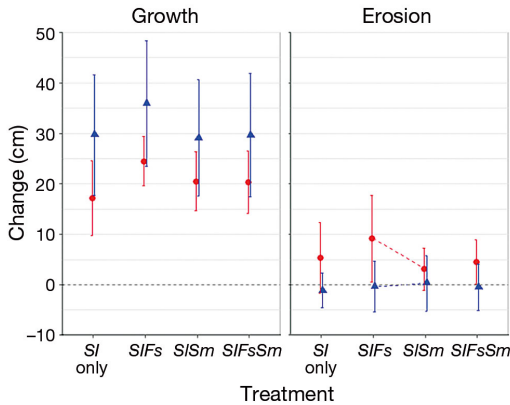
Fig. 5. Mean growth and erosion (±1 SD) of *Saccharina latis-sima* during the experiment in different treatments and years (n = 74 in 2014, n = 163 in 2015). Higher positive numbers on the erosion axis indicate that more of the blade was eroded. Symbols, colours, and abbreviations as in Fig. 4

In 2015, the mean (±SD) growth of *Saccharina* was 31 ± 12 cm, compared to 20 ± 7 cm in 2014 (Fig. 5). The maximum individual growth observed was 70 cm (2015). The age of the thalli influenced growth rate (Table 2), with mean growth of those that were <1 yr old being higher than those >1 yr old (Fig. S3). Because of this, the mean growth rates stated above may be somewhat influenced by the fact that the thalli which could be measured for growth in 2015

Table 2. Results of statistical analyses carried out to examine the effect of treatment and year (Yr) on *Fucus serratus* (*Fs*), *Saccharina latis-sima* (*Sl*) and *Sargassum muticum* (*Sm*). For each predictor, the likelihood ratio (L) or chi-squared value is given, with degrees of freedom in subscript. NS: non-significant and removed from the model; (+) included in the model due to significant interaction. p-values are shown in *italics* if over the 0.05 level; all are unadjusted. R^2 values indicate the proportion of variation explained by the fixed effects in the model; the values in brackets indicate the variation explained by the model as a whole (including random effects). In cases where 'treatment' was significant, contrasts between levels close to significance are shown in the last 2 columns. Predictions from these models (as least-squares means) are presented in the Supplement at www.int-res.com/articles/suppl/m573p085_suppl.pdf. n/a = not applicable

Species and response	Starting size	Age	Predictors Year	Treat	Year × Treat	R^2	Differences between treatment levels	
Saccharina latissima								
Length	$L_1 = 6.79$, p = 0.009	NS	+	+	$L_3 = 12.1$, p = 0.007	0.46 (0.47)	Yr × *SlFs* − Yr × *SlSm* Yr × *SlFs* − Yr × *SlFsSm*	$t_9 = -2.70$, p = 0.024 $t_9 = -2.87$, p = 0.019
Weight	$L_1 = 303$, p < 0.001	NS	+	+	$L_3 = 11.4$, p = 0.010	0.84 (0.84)	Yr × *SlFs* − Yr × *Sl* Yr × *SlFs* − Yr × *SlSm* Yr × *SlFs* − Yr × *SlFsSm*	$t_9 = -2.52$, p = 0.033 $t_9 = -2.98$, p = 0.016 $t_9 = -2.56$, p = 0.031
Growth	$L_1 = 22.3$, p < 0.001	$L_1 = 8.85$, p = 0.003	$L_1 = 17.0$, p < 0.001	$L_3 = 13.1$, p = 0.004	NS	0.34 (0.34)	*SlFs* − *Sl* *SlFs* − *SlSm* *SlFs* − *SlFsSm*	$t_{12} = -3.40$, p = 0.005 $t_{12} = -3.36$, p = 0.006 $t_{12} = -2.95$, p = 0.012
Erosion	$L_1 = 6.80$, p = 0.009	NS	+	+	$L_3 = 9.59$, p = 0.022	0.26 (0.28)	Yr × *SlFs* − Yr × *SlSm*	$t_9 = 3.13$, p = 0.012
Death/loss	NS	n/a	+	+	$\chi^2_3 = 7.17$, p = *0.067*	n/a	Yr × *SlFs* − Yr × *Sl* Yr × *SlFs* − Yr × *SlFsSm*	z = 1.79, p = *0.073* z = 2.47, p = 0.014
Fucus serratus								
Length	$L_1 = 110$, p < 0.001	n/a	$L_1 = 6.94$, p = 0.008	NS	NS	0.28 (0.35)		
Weight	$L_1 = 296$, p < 0.001	n/a	+	+	$L_1 = 17.0$, p < 0.001	0.66 (0.68)	Yr × *Fs* − Yr × *FsSl* Yr × *Fs* − Yr × *FsSlSm* Yr × *FsSm* − Yr × *FsSl* Yr × *FsSm* − Yr × *FsSlSm*	$t_9 = -2.05$, p = *0.071* $t_9 = -2.29$, p = 0.048 $t_9 = -3.12$, p = 0.012 $t_9 = -3.36$, p = 0.008
Death/loss	NS	n/a	$\chi^2_1 = 5.86$, p = 0.015	NS	NS	n/a		
Sargassum muticum								
Length	$L_1 = 5.65$, p = 0.017	n/a	NS	NS	NS	0.03 (0.05)		
Weight	$L_1 = 45.8$, p < 0.001	n/a	+	+	$L_3 = 10.7$, p = 0.013	0.37 (0.63)	Yr × *SmSl* − Yr × *Sm* Yr × *SmSl* − Yr × *SmFs*	$t_9 = 2.97$, p = 0.016 $t_9 = 2.15$, p = *0.060*
Death/loss	$\chi^2_1 = 7.19$, p = 0.007	n/a	$\chi^2_1 = 5.57$, p = 0.018	$\chi^2_3 = 6.58$, p = *0.087*	NS	n/a	*SmFsSl* − *Sm* *SmFsSl* − *SmFs*	z = −2.34, p = 0.019 z = −2.24, p = 0.025

contained a higher proportion of age 0+ thalli than in 2014 (2014: 16 of 74 thalli; 2015: 62 of 163 thalli). Despite this, there was clearly also an effect of year on growth (Table 2, Fig. 5). Starting weight was negatively related to growth and erosion (Table 2).

Saccharina survival was only slightly higher in the cool year, at 83% compared to 78% in the hot year. The interaction between year and treatment was not statistically significant according to the LRT, but the results of the Wald test indicated that the effect of

year on *Saccharina* survival in the assemblage with *Fucus* (*SlFs*) was different from the effect in the assemblage with all 3 species (Table 2). *Saccharina* survival was lower in the hot year than in the cool year in the *SlFs* treatment, while it remained similar across both years in the *Saccharina*-only treatment (Figs. 6 & S5).

The nitrogen content of the *Saccharina* thalli decreased slightly throughout the summer, from 1.07 ± 0.35% (SD) in May to 0.83 ± 0.22% at the end of the experiment in August. Conversely, carbon content increased by around 3% (Fig. 7).

Fucus serratus

The majority of *Fucus* showed positive length and weight changes in both years (Fig. 4). Its end length was unaffected by treatment, but was significantly higher in the cool year across all assemblages (Table 2), although this was a small difference (Fig. 4). Year had little consistent effect on weight,
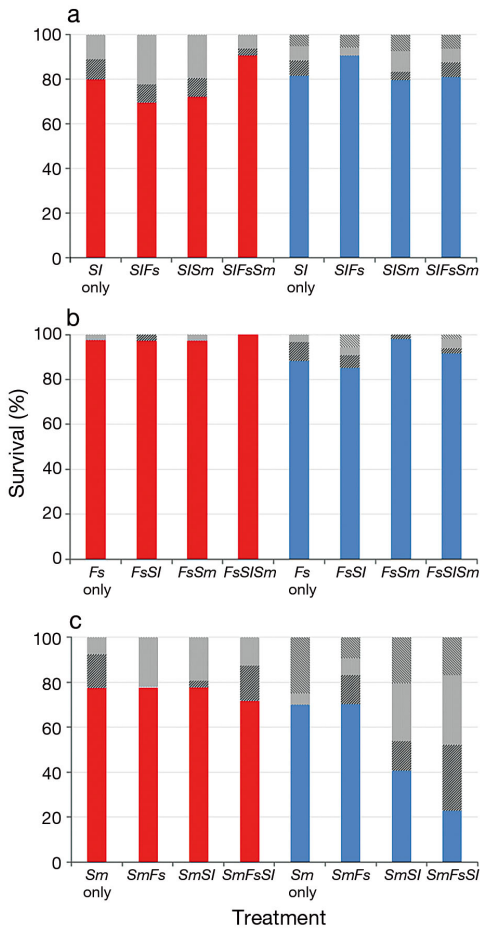

Fig. 6. (a) Total survival of *Saccharina latissima* (n = 365), (b) *Fucus serratus* (n = 360) and (c) *Sargassum muticum* (n = 360) in summer 2014 (red) and summer 2015 (blue). Coloured bars: percentage of surviving thalli in each treatment; grey areas: percentage of thalli which died or were lost during the experiment, patterned to show the split between the 2 (2014) or 3 (2015) replicates of each grid. Abbreviations as in Fig. 4
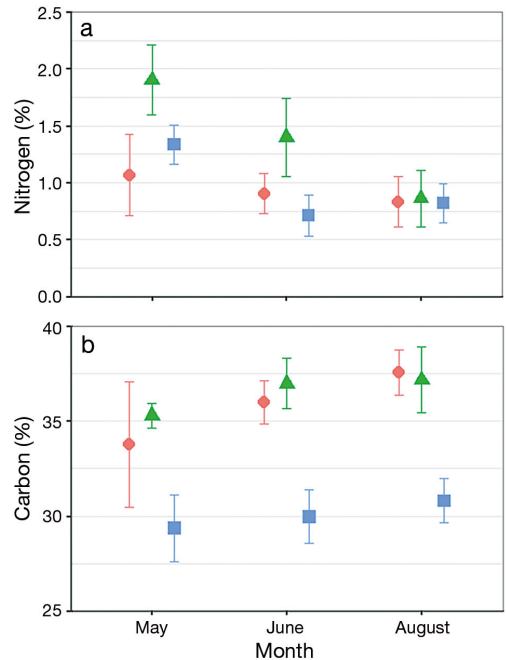

Fig. 7. Seaweed (a) nitrogen and (b) carbon content over the experiment during summer 2015. Mean percentage content of dry weight is shown for each species, ±1 SD. Red circles: *Saccharina latissima* (n = 27), green triangles: *Fucus serratus* (n = 24) and blue squares: *Sargassum muticum* (n = 22)

but interactive effects with assemblage type were present (Table 2). In the hot year, the end weight of *Fucus* was relatively similar in all assemblage types, but in the cool year it was lower when grown together with *Saccharina* than in the other assemblage types (Fig. 4). This effect was most clear in comparisons with the assemblage with *Sargassum* (*FsSm*) (Table 2, Figs. 4 & S7). *Fucus* showed the highest overall survival of the 3 species (94%). There was a slight but significant reduction in survival from 98% in 2014 to 91% in 2015 (Fig. 6), but the difference between assemblages was not statistically significant (Table 2). End length and weight were positively linked to starting length and weight, respectively, but there was no influence of the starting size on survival (Table 2).

The nitrogen content of *Fucus* decreased throughout the summer of 2015, from $1.9 \pm 0.31\%$ (SD) in May to $0.86 \pm 0.25\%$ in August. *Fucus* started with higher nitrogen content than *Saccharina* and *Sargassum*, but this difference decreased with time and by August all 3 species were similar (Fig. 7). The carbon content of *Fucus* was much higher than *Sargassum*, and increased throughout the summer (Fig. 7).

Sargassum muticum

The *Sargassum* thalli were generally in poor condition at the end of the experiment in both years; many had lost some side branches and air vesicles and were no longer buoyant. We did not observe receptacles as might be expected for this time of year in this area, and the advanced state of the decline suggested that they would not become reproductive at all. To ensure that this was not because of the experimental treatment, we visited naturally occurring *Sargassum* on the surrounding islands, and found it to be in a similar condition.

There was no difference in length between the assemblages or years (Table 2), with the thalli usually increasing by around 10 to 25 cm (but with large variation; Fig. 4). Weight change, however, showed significant interactive effects of year and treatment (Table 2). For the *Sargassum*-only, *Fucus* and 3-species assemblages (*Sm*-only, *SmFs*, *SmFsSl*), weight change was always low, but in the assemblage with *Saccharina* (*SmSl*), weight change was higher in the hot year compared to the cool year and other treatments (Figs. 4 & S9).

Sargassum survival was significantly higher in the hot year (76% in 2014 compared to 52% in 2015; Table 2). It was also always lowest in the assemblage

with both native species, significantly lower than the assemblages where *Sargassum* was alone or with *Fucus* only (Table 2, Fig. 6). In contrast to the other 2 species, *Sargassum* survival was positively linked with starting weight (Table 2); the predictions of the model are that in 2015, a thallus in the *Sargassum*-only treatment had a 24% chance of being lost if weighing 10 g, 33% if 20 g, and 44% if 30 g (parameter estimate = 0.046, SE = 0.017).

In 2015, the mean nitrogen content in *Sargassum* was $1.34 \pm 0.17\%$ (SD) at the start of the experiment in May, but this rapidly decreased by June and stayed low (Fig. 7). The nitrogen content in June 2014 was similar to June 2015 ($0.7 \pm 0.1\%$ in 2014; $0.71 \pm 0.18\%$ in 2015). As with all the species, the carbon content of *Sargassum* increased through the summer, but was consistently 5 to 7% lower than *Fucus* and *Saccharina* (Fig. 7).

DISCUSSION

Temperature

Relatively short, extreme temperature events put acute stress on cold-adapted seaweeds, in addition to the chronic stress of gradual warming (Brodie et al. 2014). If extremely warm summers become more frequent, seaweed ranges and abundances may be affected more severely, more quickly or over a wider area than predicted from changes in mean temperature alone (e.g. Smale & Wernberg 2013). In the present study, conditions in 2014 had a substantial negative impact on *Saccharina*, with moderate warming from May and a short hot period later sufficient to reduce *Saccharina* growth and increase blade erosion until there was almost no net gain in weight or length over the summer. We expect that high temperature was the main cause of this, as high temperature alone (20°C) has been shown to cause tissue deterioration, loss of pigments and reduced net photosynthesis in *Saccharina* from southwestern Norway (Andersen et al. 2013). Other temperature experiments have shown similar negative effects, with blade weakness, tissue loss and mortality increased at 18 to 20°C, and total mortality at 21 to 23°C (Bolton & Lüning 1982, Andersen et al. 2013, Simonson et al. 2015). In contrast, average summers in southwestern Norway (such as 2015) provide near-ideal conditions for *Saccharina*, which has optimum growth at 10 to 15°C (Lüning 1990, Andersen et al. 2013). The elongation rate for *Saccharina* in 2015 was on average 0.4 cm d^{-1}, which is similar to a former study of

tagged individuals (around 0.5 cm d^{-1}) during a normal summer (Sjøtun 1993).

In contrast to *Saccharina*, the different summer conditions did not have a large effect on *Fucus* and *Sargassum.* Regardless of whether it was a cool or hot summer, *Fucus* had net weight gain and high survival. *Fucus* exhibits optimum growth at only slightly higher temperatures than *Saccharina* (15°C), but can survive at temperatures up to 25°C (Lüning 1990), making it better adapted for surviving summer heatwaves. Intertidal *F. serratus* populations still persist in northern Spain, despite a reduction in abundance associated with warming (Duarte et al. 2013), and in the British Isles, Hawkins & Hartnoll (1985) also reported that intertidal *Fucus* appeared undamaged after a hot summer, while *Saccharina* showed signs of damage or mortality. The slightly lower elongation of *Fucus* observed in the hot year indicates that high temperature had some negative effect, but the elongation rates (around 0.35 cm wk^{-1} in 2014 and 0.50 cm wk^{-1} in 2015) are comparable to other locations in the NE Atlantic (Knight & Parke 1950 and references therein).

Sargassum was expected to perform better in the warmer year because its distribution in Europe stretches south to Morocco (Engelen et al. 2015) and its growth rate increases with temperature to at least 25°C (Norton 1977, but also see Sfriso & Facca 2013). Although survival improved under hotter conditions, growth and general condition were similarly poor in both years. Compared to other locations, the change in length was low and senescence was quite advanced by mid-August (Engelen et al. 2015; also compared to some other local sites in Norway). This, along with our field observations, suggests that *Sargassum* is limited by a factor other than temperature at sheltered sites. Possibilities for this include brown filamentous algal epiphytes, which may cover subtidal *Sargassum* in early summer at sheltered sites in Ireland, Scotland and Norway (Baer & Stengel 2014, Engelen et al. 2015, authors' pers. obs.); however, the thalli used in the present experiment were relatively clean. Nutrient limitation is another possible explanation (see later discussion).

Competition

Competitive interactions can be a major determinant of the composition of canopy-forming brown algae on the shore (Hawkins & Hartnoll 1985, Hawkins & Harkin 1985). The results of the present experiment are consistent with the hypothesis that

these interspecific competitive interactions may change under higher temperatures. In the cool summer, *Saccharina* was the superior competitor to *Fucus* and *Sargassum*, with the highest length and weight gains. On the other hand, when *Saccharina* was under temperature stress its growth and ability to maintain its blades were reduced. Under these conditions, *Fucus*, and to a lesser extent *Sargassum*, tended to be more successful in the treatment with *Saccharina* than they were in the cool year, demonstrating that the competitive effect of *Saccharina* was weaker. *Fucus* became the most successful, gaining the most weight. The experiment did not assess which resource was being competed for, but as the thalli already had attachment space and ambient nutrients were low throughout the summer, light is most likely. This is usually the primary resource for which competition occurs in the sublittoral (Lüning 1990).

The strongest interactions observed were those between the native seaweeds. *Saccharina* had a strong competitive effect on *Fucus*, with *Fucus* gaining much less weight when *Saccharina* was in good condition. This was expected, as *Fucus* is capable of growing deeper than normally observed but is competitively excluded by kelps (Kain & Jones 1975, Hawkins & Hartnoll 1985). However, *Fucus* also affected *Saccharina* in 2 ways. The first was temperature-dependent, as shown by the differences in erosion, weight and mortality of *Saccharina* between the cool and hot summers in the assemblage with *Fucus*. A possible explanation is interference competition. When water temperatures are maintained at ≥18°C, the blades of *Saccharina* become considerably more fragile (Simonson et al. 2015), potentially making it more susceptible to abrasion and tearing by the leathery, serrated *Fucus* branches. The second effect was increased growth of *Saccharina* in the *SlFs* assemblages, which was independent of temperature. High biomass of *Fucus* around the *Saccharina* thalli may alter conditions (e.g. light levels), causing the kelp to allocate resources to elongation rather than thickening or widening of the blade. Field studies have shown that light or wave exposure can affect blade thickness, width and growth allocation in kelps (Sjøtun & Fredriksen 1995, Wing et al. 2007). The effects of *Fucus* on *Saccharina* were not evident in the treatment with all 3 species, possibly due to the inclusion of *Sargassum* lowering the density of *Fucus* and *Saccharina*.

Britton-Simmons (2004) found that *Sargassum* can negatively affect sublittoral macroalgae by exploitative competition for light. However in the

present study, *Sargassum* had no more effect than intraspecific competition between *Fucus* and *Saccharina*. Strong & Dring (2011) also found no effect of *Sargassum* on *Saccharina* growth or erosion, and Vaz-Pinto et al. (2014) found no negative effect of *Sargassum* on growth of the native fucoid *Cystoseira humilis*. The poor condition of *Sargassum* at the end of the present experiment may have limited its competitive ability, but this would not apply to the other studies. For *Saccharina*, it may be that adaptation to low light (Fortes & Lüning 1980) makes adult thalli relatively resistant to shading by *Sargassum* when in the upper subtidal. But it may also be that competition between adult thalli is not a critical factor in explaining the success of *Sargassum* (Vaz-Pinto et al. 2014).

The competitive effects of *Fucus* on *Sargassum* were similar to the effects of intraspecific competition in *Sargassum*. Vaz-Pinto et al. (2014) also found no competitive effect of the fucoid *C. humilis* on *Sargassum* growth. However, *Saccharina* appeared to have a negative influence on *Sargassum* survival under average temperatures. This differs from the findings of Strong & Dring (2011), who found no competitive effect of *Saccharina* on *Sargassum* even at high densities, and suggested that the presence of *Saccharina* actually benefitted *Sargassum* by reducing intraspecific competition. This contradiction may be partly explained the poor state of *Sargassum* by the end of the present study, which probably reduced intraspecific competition to low levels. Growth conditions for *Sargassum* vary locally on the southwestern coast of Norway, and its condition appears to be better in places with strong tidal currents. In Ireland it also is less successful at sheltered sites (Baer & Stengel 2010). Repeating the experiment at locations with higher water movement would be useful future work, to clarify how local conditions affect competitive interactions.

Sargassum survival was always lower in the treatment with both native species, suggesting that *Fucus* and *Saccharina* have a combined negative effect on *Sargassum*. Because only the trend in survival was statistically significant, and the difference was relatively small in the hot summer, more work must be done before drawing definitive conclusions. However, it does fit with the idea that diversity may reduce vulnerability to invasion on a local scale (Elton 1958). Theoretically, more native species use resources more completely, instead of leaving them available for non-native species (Stachowicz & Tilman 2005). This may be particularly important if environmental conditions change, making some native species less effective in using resources, as happened with *Saccharina* in our study during the hot summer. If resource availability is the key factor rather than diversity per se, invasion resistance could occur with a smaller number of native species as long as they are efficient at coping with the variability in resources (Dunstan & Johnson 2007).

Nutrients may also play a role in seaweed competitive relationships. The decreasing internal nitrogen content in all 3 species from May to August reflects low external nitrogen availability during summer in the study area (Strömgren 1986, Pedersen & Borum 1996), with an average nitrogen content of around 0.8% in August suggesting that all 3 species were nitrogen limited during this season (Dean & Jacobsen 1986, Duarte 1992, Pedersen & Borum 1996). Internal nitrogen levels were relatively low compared to other published values, such as *Fucus*: 2.5% in May and 1 to 1.3% in August (Scotland; Brenchley et al. 1998); *Saccharina*: 1.6 to 2.6% in May and 1 to 1.6% in July (Denmark and southwestern Norway; Sjøtun 1993, Nielsen et al. 2014); *Sargassum*: 1 to 2% in June through August (Denmark; Wernberg et al. 2001) and 1.8% in August (Portugal; Vaz-Pinto et al. 2014).

Low summer nutrients may be particularly limiting for *Sargassum* due to its phenology. *Saccharina* and *Fucus* are perennial and may store nitrogen, so although growth is fastest early in the year when nutrient levels are higher, it can continue for some time in low nutrient conditions during the summer (Strömgren 1986, Sjøtun et al. 1993, Brenchley et al. 1998, Nielsen et al. 2014). On the other hand, *Sargassum* is pseudo-perennial, with thalli growing rapidly from a basal holdfast in February or March until receptacle formation in mid-July, followed by senescence in late August or September (in southwest Norway; timing varies by location; Engelen et al. 2015). This pseudo-perennial strategy can lead to a competitive advantage in some environments, as rapid growth and air vesicles allow it to form a floating layer, shading algae below (Britton-Simmons 2004). It also allows *Sargassum* to avoid expending energy in maintaining a large thallus over long periods, which may explain its lower carbon content than perennial brown seaweeds (Wernberg et al. 2001, this study). However, in southwestern Norway, this strategy means that *Sargassum* must quickly gain considerable biomass and become reproductive during a period of very low water nutrients. Fast-growing algae are also more susceptible to nutrient depletion; for example, the minimum nitrogen content for growth is 0.71% in the fast-growing *Ulva lactuca*, compared to 0.55% in *Fucus vesiculosus* (Pedersen &

Borum 1996). A nitrogen content of 0.7% suggests that *Sargassum* was severely nitrogen limited by June, which may explain its poor condition and have led to a competitive disadvantage compared to *Fucus* and *Saccharina.*

Limitations and implications

This experiment only examined competition between adult thalli, as adult competition for light is expected to be important in *Saccharina*–*Sargassum* interactions (Strong & Dring 2011). However, competition varies depending on the size and life stage of seaweeds (Olson & Lubchenco 1990). *Saccharina* and *Fucus* tend to recruit in autumn and winter, when *Sargassum* biomass is low and infertile. The presence of *Sargassum* is therefore unlikely to affect recruitment of these species in the NE Atlantic, as it can in regions where kelp recruitment and *Sargassum* peak biomass periods coincide (e.g. Ambrose & Nelson 1982). However, *Saccharina* and *Fucus* maintain a perennial canopy which could reduce recruitment of *Sargassum* (Sánchez & Fernández 2006) or survival of recruits through shading (Britton-Simmons 2006, Vaz-Pinto et al. 2012). These effects are also likely to be important in determining the success of *Sargassum* (Vaz-Pinto et al. 2014). Competition can also be influenced by the density of individuals (e.g. Strong and Dring 2011), which was not manipulated in the present study.

This experiment was carried out in the field and took advantage of a natural event where 2 successive years had contrasting thermal conditions. An advantage of this is that it allowed us to observe effects on the algae in the presence of all their natural interactions; however, a limitation is that factors other than temperature may also have varied between the 2 years. Photosynthetically active radiation (PAR) and UV levels may have been higher during 2014, which can affect macroalgae additively or interactively with temperature (e.g. Tait & Schiel 2013, Xiao et al. 2015). However, the thalli were sublittoral and mean cloud cover was intermediate in both years, which would have reduced the influence of this factor. Since the first year had the highest temperatures, this may also have influenced the results the following year; for example, by causing acclimatisation of the seaweeds to higher temperatures, or by influencing the rest of the community (e.g. grazer populations). However, we did not observe large differences in grazers, and the condition of the 3 species under the different tempera-

tures was consistent with existing literature, as discussed previously. A final consideration is that *Sargassum* 'survival' in our experiment applied to the laterals, but the holdfast is perennial and may survive to grow again the next year(s) after poor conditions.

Despite these limitations, our observations can be used with existing studies to provide some insight into the effects of hotter summers in the sublittoral fringe. The negative effects on *Saccharina* could lead to reduced abundance in the uppermost subtidal and/or sheltered areas where the water warms more (e.g. Filbee-Dexter et al. 2016), especially if additional stressors are present (Moy & Christie 2012, Andersen et al. 2013). Widespread declines in *Saccharina* have already occurred along the Norwegian coast during a period of hot summers from 1996 to 2002 (Moy & Christie 2012), and marine heatwaves can affect large areas of the NE and NW Atlantic where *Saccharina* is an important component of sheltered seaweed communities (Mills et al. 2013, Joint & Smale 2017). However, temperature ecotypes exist within *Saccharina* (Lüning 1975, Gerard & Du Bois 1988) and it may be able to acclimatise (Andersen et al. 2013), suggesting the effect of temperature may not be uniform. *Fucus* coped well with the hot summer. It can be limited from the subtidal by competitive interactions with kelp (Kain & Jones 1975, Hawkins & Hartnoll 1985) and may therefore become more common in the upper sublittoral where *Saccharina* has declined, as has occurred with warming in Nova Scotia (Filbee-Dexter et al. 2016). It seems unlikely that warming will directly benefit adult *Sargassum* at sheltered sites in this area, as their condition appears to be limited by another factor, but a reduction in *Saccharina* could improve survival of adult *Sargassum* and create physical gaps for *Sargassum* recruitment. However, for a more reliable indication of what may happen with *Sargassum* in this region, research should be conducted examining the causes of its patchy success.

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Errata for
Codium fragile subsp. *fragile* and *Sargassum muticum*: Non-native seaweeds in a changing environment
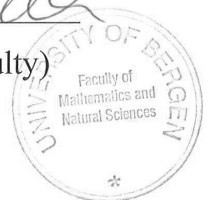
Caroline Susanne Armitage

Thesis for the degree philosophiae doctor (PhD)

at the University of Bergen

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(signature of candidate)

(signature of faculty)

04.10.2017

Errata

Page 65 "This estimated the mean ambient nitrate (± standard deviation) during the experimental period was 7.16 ±1.57 µg l^{-1} (0.12 µmol l^{-1}) at the farm sites, and 5.70 ±1.17 µg l^{-1} (0.092 µmol l^{-1}) at the control sites." – Should be corrected to "This estimated that the mean ambient inorganic nitrogen (ammonium, nitrite and nitrate; ± standard deviation) during the experimental period was 7.16 ±1.57 µmol l^{-1} at the farm sites, and 5.70 ±1.17 µmol l^{-1} at the control sites."