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)

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Abstract

Codium fragile subsp. *fragile* (hereafter *C. fragile*) is a widespread non-native chlorophyte. Experimental and observational approaches were used to examine the influence of temperature on its growth and local distribution in southwestern Norway, where it has been established for over 80 years. Growth was measured in laboratory experiments at current and predicted seasonal minimum and maximum seawater temperatures, and under natural conditions by recording length over one year in the field. The results indicated that if temperatures increase, *C. fragile* growth rate may increase in spring, but not in mid-autumn/winter due to low light. Thallus fragmentation was common in autumn/winter in the field, but very rare in the winter-temperature laboratory experiment, indicating that low temperature is not the only driver. Growth occurred at temperatures which are reported as too low for growth in other locations, suggesting that *C. fragile* can acclimatise/adapt to local temperature regimes. Local distribution was examined by surveying abundance using categories based on number of thalli and their arrangement (scattered/patch/zone) at 46 stations from 2011-2016. *Codium fragile* colonised the stations relatively often, but was frequently lost from sites with only bedrock compared to sites with stony substratum present (cobbles/boulders). It was more likely to be consistently present at sites with stones, and could form dominant patches of canopy in the upper sublittoral on this substratum. An increase in number of stations with *C. fragile* present was seen after two mild winters, suggesting that higher minimum temperatures may increase site occupancy of *C. fragile* in this region.

Running head: *Codium fragile* and temperature

Introduction

Coastal ocean temperatures are predicted to rise in the future (Wong et al. 2014), which may lead to changes in species distributions. Macroalgae are particularly likely to be affected because sea surface temperature is an important factor controlling their growth, survival and reproduction (van den Hoek 1982). In the Northern Hemisphere, declines and local disappearances are predicted to occur along the southern borders of macroalgal species distributions, while areas further north may become colonised as temperatures rise (Müller et al. 2009). Changes in ranges of seaweeds and the composition of macroalgal communities has already been documented in temperate zones; for example, increasing temperature has been associated with a change in the relative abundances of temperate and tropical seaweeds in Japan (Tanaka et al. 2012) and canopy-forming brown algae in the British Isles (Yesson et al. 2015). Changes in the ranges of canopy seaweeds have also been associated with temperature increases in southern Europe and Australia (Wernberg et al. 2011; Duarte et al. 2013; Smale and Wernberg 2013). Non-native species are likely to undergo range changes in response to temperature too, with their effects on native communities interacting with the effects of climate change (Occhipinti-Ambrogi 2007). As macroalgae are important habitat-forming organisms and primary producers (e.g. Norderhaug et al. 2003; Christie et al. 2009; Migné et al. 2015), an increase in negative effects of non-native macroalgae on native macroalgae (Thomsen et al. 2016) could have a large impact on coastal ecosystems. How individual seaweed species will react to increased temperature, how this will affect communities, and how the impact of non-native seaweeds will interact with these effects, are pressing questions in this period of global change.

Predictions suggest that southern-mid Norway will be one of the nine regions of the Atlantic Ocean where phytobenthos will be most affected by temperature changes (Müller et al. 2009). Norway has approximately 100,000 km of coastline, spanning 13° latitude (SSB 2016), which is a huge habitat area for seaweeds, and contains a macroalgal flora of just under 500 species. Under warmer conditions, new species may colonise the Norwegian coast by radiating naturally from southern populations, while species with a present distribution limit in southern Norway may expand their distribution or abundance in response to the rise in temperature. This has already contributed to changes in macroalgal communities in southwest Norway (Husa et al. 2008; Sjøtun et al. 2015). Simultaneously, conditions may become poorer for the dominant native kelps which are adapted to cooler waters (Fortes and Lüning 1980). Unusually hot summers in southern Norway have been associated with large declines and poorer condition of the kelp *Saccharina latissima* (Moy and Christie 2012, Armitage et al. in press), and kelps are expected to continue to decline towards the southern edge of their ranges as mean temperatures, the frequency of extreme

summer weather, and the impact of warm-adapted non-native species increases (Wong et al. 2014).

A common non-native alga in southwest Norway is the chlorophyte *Codium fragile* subsp. *fragile* (Suringar) Hariot, a high-profile invader which has spread and been studied globally (Trowbridge 1998; Davidson et al. 2015). It has been present in Norway since at least 1932 (Armitage and Sjøtun 2016a) and is categorised as a high impact species according to the Norwegian blacklist (Gederaas et al. 2012). Although patchily distributed, it can form the dominant sublittoral canopy at some sites, and is mainly restricted to below mean low water in this region (Armitage et al. 2014). *Codium fragile* subsp. *fragile* is not associated with large negative impacts on abundances or diversity of macroalgae-associated invertebrates or epiphytic macroalgae, but can affect the community composition of these groups (Schmidt and Scheibling 2006; Jones and Thornber 2010; Armitage and Sjøtun 2016b), and influence detritus cycling (Krumhansl and Scheibling 2012). Once established, it may occupy space and prevent reestablishment of kelp (Levin et al. 2002, Scheibling and Gagnon 2006).

The thallus of *C. fragile* consists of a mat-like holdfast and spongy, cylindrical, dichotomous branches to around 15-35 (-50) cm long (Brodie et al. 2007). Like other species in the genus *Codium*, it has a coenocytic structure where the thallus is formed from a few large cells, highly-branched into siphons. The siphons tangle tightly to form the medulla of the thallus, and have swollen tips (utricles) which face outwards to form the thallus surface (Electronic Supplementary Material 1). *Codium* species have a diplontic life cycle, and produce anisogametes in gametangia on the utricles. Most species produce male and female gametes and have dioecious thalli, although parthenogenesis and monoecious thalli are recorded in several species (Prince and Trowbridge 2004, Brodie et al. 2007). North Atlantic *C. fragile* subsp. *fragile* is thought to do this exclusively, with gamete formation in late summer/autumn producing only female gametes, which develop by parthenogenesis (Trowbridge 1998). Asexual reproduction in *C. fragile* subsp. *fragile* may also occur through thallus fragmentation. This is a process whereby the tangle of medullary siphons starts to unravel, which can lead to length reduction if at the tip of a branch or branch breakage if lower down (Fralick and Mathieson 1972) (see illustration in Electronic Supplementary Material 1). The branches or utricles which break off can survive, reattach to substratum and form new thalli (Yotsui and Migita 1989, Trowbridge 1998, Scheibling and Melady 2008). Branch fragments may drift long distances as they float in good light conditions (Gagnon et al. 2014), and can spread parthenogenetic gametes if reproductive as well as forming new thalli by regrowth. The rapid spread of *C. fragile* subsp. *fragile* (Trowbridge 1998) has likely been facilitated by these effective propagation methods.

Codium fragile subsp. *fragile* (hereafter *C. fragile*) is occasionally referred to as a warm-temperate alga (Fralick and Mathieson 1973), but its distribution extends from the subtropics to the northern part of the cold temperate region in the NE Atlantic, and Newfoundland in the NW Atlantic (Stellander 1969; Trowbridge 1998; Matheson et al. 2014). Despite this, there are indications that low temperatures may limit its success. In the Gulf of Maine and Scotland, *C. fragile* was observed to be most abundant at sites with locally higher temperatures (Carlton and Scanlon 1985, Trowbridge and Todd 1999, Mathieson 2003, and references therein). In Norway, *C. fragile* is not present in the coldest areas, being absent or rare to the south on the Norwegian Skagerrak coast (Husa et al. 2013) where water temperature regularly reaches – 1 °C (IMR 2016), and rare in and around the mouths of large fjords (Fægri and Moss 1952) where surface water has low temperatures and salinities (Sjøtun et al. 2015). Low air temperatures may limit its colonisation of emergent substrata in the littoral zone (Trowbridge 1998), and low water temperatures have been proposed as a cause of fragmentation (Fralick and Mathieson 1972). Although it can survive at 0 °C (Lüning 1984), its optimum growth occurs at much higher temperatures (24 °C) (Fralick and Mathieson 1973; Hanisak 1979a) and it requires temperatures of 12-15 °C for gametangia formation (Churchill and Moeller 1972). These observations suggest that if there is an increase in mean ocean temperature, or if the frequency of years with hot summers and mild winters increases in Norway, then *C. fragile* is a species that would benefit. These changes may lead to an increase in *C. fragile* abundance through faster growth rates, increased survival and a longer gamete-production period, which could result in increased colonisation rates and/or persistence at sites.

Within its introduced range, the majority of work on *C. fragile* and temperature has been carried out in the NW Atlantic. However, temperature experiments have shown variable results depending on the source population of the *C. fragile* thalli (Malinowski 1974; Trowbridge 1998), with a possible explanation being local adaptation or acclimatisation to different temperature regimes. It is possible that *C. fragile* may therefore show different responses to warming in different regions. There is also relatively little published research on the distribution of *C. fragile* in the NE Atlantic outside the British Isles, where it is mostly littoral (Chapman 1999), leaving questions about its distribution and persistence in sublittoral NE Atlantic habitats.

The present study has therefore investigated *C. fragile* abundances and growth rates in south-west Norway using three different approaches and time scales: (1) short-term laboratory experiments to test whether growth rates increase at predicted future summer and winter temperatures; (2) measurements of growth rate in the field over one year, to measure the seasonal cycle of growth, and examine how it relates to temperature and light; and (3)

semi-quantitative recordings of *C. fragile* abundance at 46 stations over 5 years, to record how local *C. fragile* abundance changes through time, how persistent it is once established, how often successful colonisation of stations occurs, and test if these are related to substratum or ambient temperature variation.

Materials and Methods

Study area

The coast of southwestern Norway consists of an archipelago-type landscape interspersed with fjords. It is within the cold-temperate biogeographic zone (Lüning 1990), with a shoreline of mostly hard substratum (sloping bedrock interspersed with falls of loose boulders and cobbles). This provides a large habitat area for seaweeds, with dominant canopy species being kelps and fucoids. The work of the present study was carried out in a 30 km range among islands to the south of Bergen (between 60.073900 latitude, 5.217233 longitude and 60.301217 latitude, 5.166733 longitude; Fig. 1). All genetically sequenced samples of *C. fragile* from this study area have been the invasive subspecies, subsp. *fragile* (Armitage and Sjøtun 2016a).

Along this stretch of coast, the mean monthly sea surface temperature (SST; here referring to the temperature at 1 m depth) is highest in August and coldest in February/March. Between 1980 and 2009, mean SST was 5 °C in February (3.0 and 7.3 °C in the coldest and warmest Februarys, respectively) and 15.4 °C in August (12.8 and 18.4 °C in the coldest and warmest Augusts, respectively) (IMR 2016). Water temperature in the upper few metres is mainly determined by local meteorological conditions, while the temperature beneath is more constant and controlled by conditions in the Norwegian coastal current, which runs northwards from the Skagerrak and is joined by warmer Atlantic water to the north in Mid-Norway (Bakketeig et al. 2016). This current has salinities of around 31-32 at the surface, but this may be reduced in the areas around the mouths of narrow large fjords (e.g. Sognefjord; IMR 2016). Water nutrients in the area are very low during the summer months, and follow a seasonal pattern of higher ambient nutrient content from September to March (Strömgren 1986, Pedersen and Borum 1996, Armitage et al. in press).

Abiotic data

A temperature logger (Tinytag Aquatic 2, Gemini data loggers, Chichester, UK) recorded seawater temperature every 2 hours at 1 m depth between January 2014 and September 2016 at Espegrend Marine Biological Station (Fig. 1). Additional SST information was collected from regional hydrographic stations (Indre Utsira and Sognesjøen, 1-3
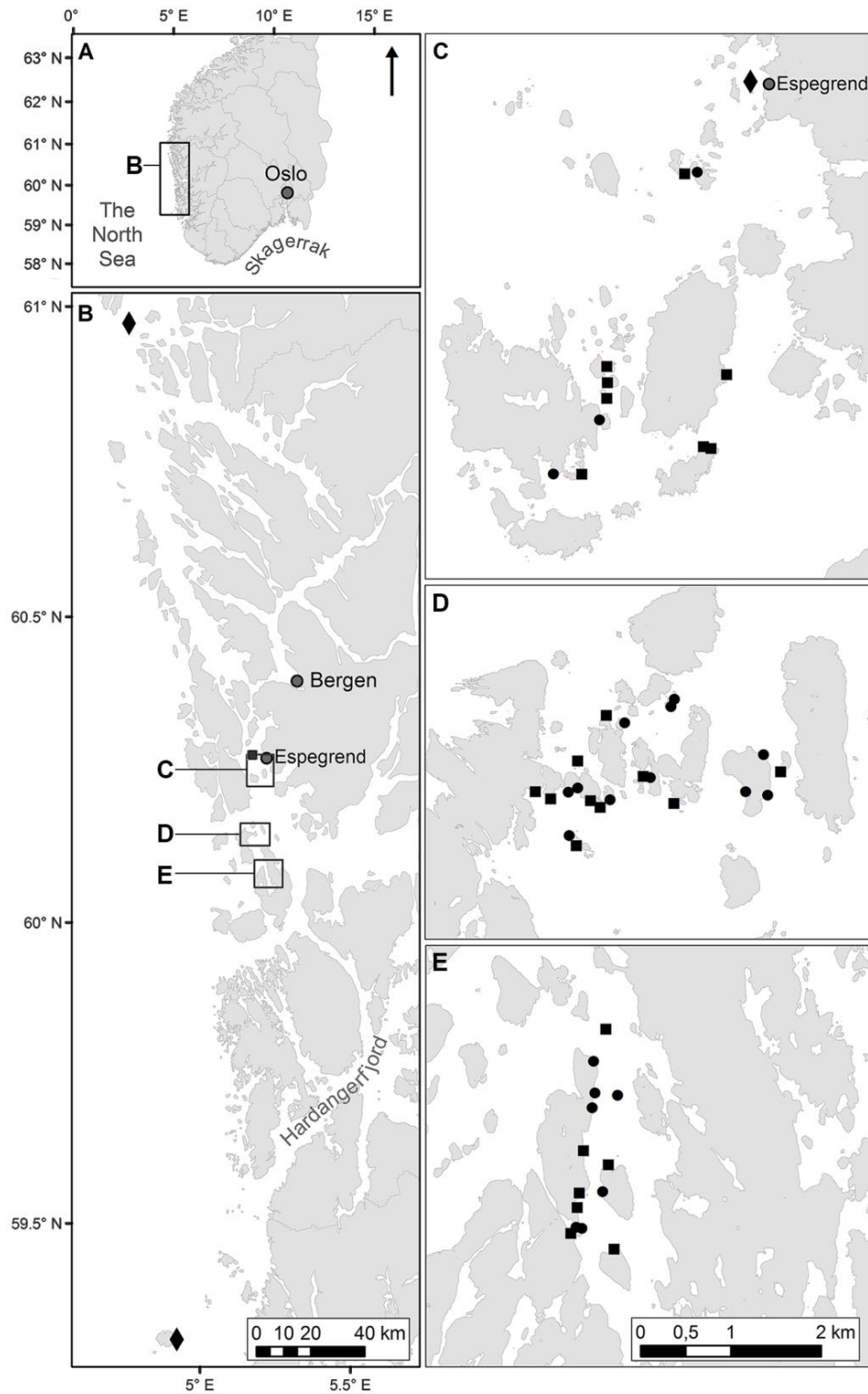
Fig. 1 Maps of the study area. Map A shows southern Norway. Map B shows the locations of Bergen, Espegrend Marine Biological Station, the Institute of Marine Research fixed hydrographic stations (black diamonds), and the fjord where CTD data from the University of Bergen was recorded (Raunefjord, black square). Maps C, D and E display the locations of the 46 survey stations (circles, stations with stony substratum present; squares, bedrock stations). Map C also shows Espegrend, and the location of the continuous temperature logger (black diamond)

measurements per month) run by the Institute of Marine Research (IMR 2016), and a CTD dataset taken in Raunefjord by the University of Bergen (UiB; 1-5 measurements per month) (Fig. 1). The latter also recorded salinity. Information about light levels was extracted from a dataset of mean daily global solar irradiance at the surface in Bergen between 1965-2013, provided by the Geophysical Institute (GFI) at the University of Bergen.

(1) Growth of Codium fragile *under controlled conditions*

Laboratory experiments were run at temperatures based on predictions for the region in Müller at al. (2009). By the years 2080-2099, mean August SST is predicted be around 2 - 2.5 °C higher than 1980-1999 levels (Müller et al. 2009), resulting in a mean of 16.3 – 18.0 °C in this study area (mean August SST 1980-89 = 14.3 °C, 1990-1999 = 15.5 °C; IMR 2016). February SSTs in 2080-2099 are expected to be 1.5 – 2 °C higher than 1980-1999 levels (Müller et al. 2009), resulting in a mean of 5.7 - 7.5 °C (mean February SST 1980-89 = 4.2 °C, 1990-1999 = 5.5 °C; IMR 2016). Thus the summer temperatures chosen for investigation were 15 °C and 18 °C, and the winter temperatures were 5 °C and 7 °C. Recent mean SSTs are similar to the low temperature used in the winter experiment, and slightly higher than the low temperature used in the summer experiment (2010-2016 mean February SST = 5.3 °C, mean August SST = 16.4 °C; IMR 2016).

The winter experiment was carried out once, and the summer experiment twice. *Codium fragile* thalli for the winter experiment were collected during the coldest period of the year (February 2016), and those for summer experiment in the warmest period (September 2015, August 2016). For each experiment, 40 thalli were collected from around chart datum in the area surrounding Espegrend Marine Biological Station (Fig. 1), and stored in flowing seawater at ambient temperature.

The experimental setup for the summer 2016 experiment and the winter experiment was as follows. One branch, showing no signs of fragmentation, was cut from each *C. fragile* thallus, until 36 branches were collected. Each branch was then cut in half at a dichotomous branching point, giving a 'branch pair'; two similar branches, one for each of the two temperature treatments. These 72 branches were individually labelled to track which branch pair they belonged to. By using two branches from the same individual thallus to compare growth rates at different temperatures, it controls for the effect of individual differences in growth rate. Maximum length and wet weight (after lying on paper towels for 30 seconds) were recorded for each branch. The 36 *C. fragile* branch pairs were then distributed evenly between 6 experimental replicates. Each replicate consisted of 2 tanks, one at each temperature, and each holding around 50 litres (56x39x42 cm). The physical arrangement of the experiment was thus 12 tanks (6 at each temperature) arranged in pairs (a replicate).

Each tank contained 6 *C. fragile* branches, resulting in 36 branches in each temperature treatment.

The summer 2015 experiment was set up in the same way but replication level was higher – 40 branch pairs were taken from 40 thalli (80 branches), and 8 replicates were set up (16 tanks, each containing 5 branches, giving 40 branches at each temperature). Also, prior to the summer 2015 experiment the thalli were treated for 2 days in germanium dioxide to hinder the growth of benthic diatoms which grow rapidly in warm temperatures, but this was not done in the summer 2016 experiment in case it affected the *C. fragile* thalli. In all experiments, length and number of dichotomies were similar within branch pairs, and all branches used were between 63 and 167 mm in maximum length.

Within each tank, the *C. fragile* samples were spaced evenly and attached at the base so they could stand upright, and an aquarium pump was added to create water circulation. The experiment was carried out in a climate-controlled room at the University of Bergen which has constant supplies of heated, ambient and cooled seawater. The tanks all had continuous water-exchange fed by this system, and desired temperatures were achieved by mixing the various supplies. The seawater from the system was UV-treated and sand-filtered before entering the tanks, and was pumped from around 100 m depth with a salinity of 35. Water from this depth contains a relatively high nutrient concentration year-round.

The irradiance was constant within replicates as each light covered a tank-pair. Daylight fluorescent bulbs (5400K) were used (spectral range of 300-750 nm, with the vast majority emitted in the 400-700 nm visible light range; Osram 36W/954, Osram, Munich, Germany). Photoperiod was adjusted to mimic natural conditions, while intensity was kept similar. The winter experiment was kept on a 8:16 hour light:dark cycle at mean irradiance of 43 μmol m^{-2} s^{-1} (ranging between 38-53 μmol m^{-2} s^{-1}). The summer experiments were on a 16:8 light:dark cycle, with mean irradiances of 65 μmol m^{-2} s^{-1} (51-76 μmol m^{-2} s^{-1}) and 50 μmol m^{-2} s^{-1} (40-55 μmol m^{-2} s^{-1}) in the summer 2015 and 2016 experiments respectively. This difference should not cause a large difference in growth rate according to Hanisak (1979a), where the fastest decreases in growth rate were observed when irradiance was less than 30 μmol m^{-2} s^{-1}.

Because the tanks had continuous flow, water temperatures fluctuated slightly throughout the experiment. Temperature loggers in 4 of the tanks showed that the mean temperatures in the winter experiment were 7.4 ± 0.3 °C (mean and standard deviation) in the high temperature treatment and 5.1 ± 0.4 °C in the low treatment; in the summer experiment, temperatures were 17.9 ± 0.6 °C (2016) and 18.0 ± 0.3 °C (2015) in the high temperature treatment, and 15.2 ± 0.4 °C (2016) and 15.0 ± 0.3 °C (2015) in the low treatment. The experiments were run for 3 weeks, with the *C. fragile* samples measured once per week. Fragmentation was

defined by a weight loss equal or over 0.2 g, any loss in length, or by a noted observation of the thallus unravelling.

(2) Growth of Codium fragile *in the field*

In August 2014, 13 *C. fragile* thalli growing on loose stones (approximately 10 cm diameter) were collected haphazardly from a rocky shore near Espegrend Marine Biological Station (Fig. 1), at approximately 0.5 m below low tide. The thalli were measured for maximum length, tagged, and the stones they sat on attached in plastic mesh shallow baskets (approximately 10 cm high). Starting lengths of the thalli were between 105 and 303 mm (mean = 157 mm). The baskets were then lowered into the midst of a large natural sublittoral *C. fragile* patch at around 1 m below mean low water outside the field station, a relatively sheltered site. The baskets were raised every 2-6 weeks to record measurements of maximum length and comments on condition for each tagged individual for one year, until late August 2015. Maximum length was chosen as this measurement allows minimal handling and damage to the thalli; however, it means that growth could be underestimated (e.g. if the longest branch was lost). The baskets were revisited 1 year after this (August 2016) to see whether the same *C. fragile* thalli were present and whether there had been additional settlement.

(3) Local distribution of Codium fragile *through time*

Surveys of *C. fragile* abundance took place during the summers of 2011, 2014, 2015 and 2016 at 46 stations (Fig. 1). Abundance was recorded in semi-quantitative categories, by estimating the number of thalli at the station in categories (0, 1-20, 20-50, or > 50) and how they were growing (as scattered individuals amongst other algae, in patches, or in a zone >75 % of the station); this information was then combined to categorise the sites into 'absent', 'low' or 'high' abundance of *C. fragile*. If *C. fragile* formed a clear zone and more than 20 individuals were counted, or if more than 50 individuals were counted occurring in patches, *C. fragile* was recorded as 'high' abundance. If *C. fragile* was present but not forming a zone, and there were also fewer than 50 individuals present, it was recorded as 'low' abundance. This is the same system as used in Armitage et al. (2014) when the original survey (2011) was carried out, and was done because the estimate of number of thalli could be susceptible to errors. By combining the growth pattern and abundance, a coarser but more reliable picture of *C. fragile* at the site is formed. Surveys were done by the same observers in all years, from a small boat around low tide in calm weather. As observations were from the surface, only *C. fragile* that was growing within visual range was included, approximately down to 3 m in the sublittoral zone.

The stations were defined as 15-20 m long sections of shoreline, and were originally selected to give a mix of *C. fragile* abundance categories. Therefore, they do not represent the distribution of *C. fragile* on the southwestern coast of Norway, but they may be used to examine temporal changes in *C. fragile* distribution in relation to environmental changes. The 2011 data was used as the baseline for monitoring, and at this point the stations were reasonably even between the *C. fragile* abundance categories (absent, n=15; low, n=16; high, n=15), with the majority of *C. fragile* occurring in the infralittoral and sublittoral zones (Armitage et al. 2014).

From the abundance data, "colonisation" and "loss" events were calculated. This was done by categorising the *C. fragile* abundance changes at a station which occurred between two surveys: changes from absence to presence at a station as "colonisation", and from presence to absence as "loss". "Persistence" is used to describe whether *C. fragile* was present at a station in all surveys. It should be noted that these changes were studied in a relatively small area (<30 km) which *C. fragile* originally colonised over 80 years ago; therefore "colonisation", "loss" and "persistence" are here used to describe small scale patterns between and within habitat patches, rather than large-scale patterns in range change.

Wave-exposure and substratum at the stations was originally recorded in the 2011 survey (Armitage et al. 2014). Wave-exposure at the stations ranged from sheltered to semi-exposed, with none facing open sea. According to a relative measure of cartographic exposure, most of the sites were towards the sheltered end of the range (Armitage et al. 2014). Salinity at the stations was expected to be similar to the coastal current and with few differences between stations, as the stations were located on islands towards the outer edges of the coast away from fjords with large freshwater outputs. The inclination at the stations was normally from around 20 - 70°, and there was little to no sedimentation at the depths surveyed, with the vast majority of visible substratum being rock. This was either bedrock or stony substratum ('bedrock' here being fairly smooth consolidated bedrock, and 'stony' being heaps of loose cobbles or small boulders, most with diameters from 5 cm to 50 cm). Station substratum and cartographic wave-exposure were unrelated (Armitage et al. 2014). The results of Armitage et al. (2014) indicated that the stations with substratum dominated by stones and stations which had both bedrock and stony substratum present were similar with respect to *C. fragile* abundance when recorded using abundance categories, thus these stations were grouped into one category ("stony") for the present study (bedrock, n=25; stony, n=21). Five stations were excluded from the original 51 used in Armitage et al. (2014) due to difficulty relocating the station precisely or it being used for other experiments. In addition, during the re-surveys it was observed that two of the stations

originally recorded as having bedrock substratum actually had some stones sublittorally, thus their substratum category was changed for the present analysis. Station data are available in Electronic Supplementary Material 2.

Analysis

Analysis was carried out in R (R Development Core Team 2016). In all cases, mixed models were used to allow the inclusion of random effects, which take into account when multiple observations are not fully independent (e.g. they are from the same thallus, or the same replicate tank). Linear mixed effects models were used for continuous response variables (R package *nlme*, Pinheiro et al. 2015) and binary logistic regression for binary response variables (generalised linear mixed effects models; r package *lme4,* Bates et al. 2015). Significance testing of predictors was done using likelihood ratio tests (Zuur et al. 2009). Post-hoc tests to find significant differences between variable levels were done by pairwise multiple comparisons of least squares means, adjusted with Tukey HSD (R package *lsmeans,* Lenth 2016). R^2 values are provided as conditional (R^2c) which shows the variance explained by the main effects, and marginal (R^2m) which also includes the random effects (R package *MuMIn*, Barton 2016). Plots were created using R, the R package ggplot2 (Wickham 2009), and Microsoft Excel.

Differences in growth between high and low temperatures in the laboratory experiment were tested using the relative growth rates (RGR), calculated from length and weight separately. The formula used to calculate RGR was (($\log_e$(*size in week 3*) – $\log_e$(*size in week 0*))/*number of days*) (Lüning 1990). Temperature (high or low) was a categorical predictor, and thallus and replicate were included as nested random effects. Branch pairs were removed from the dataset prior to analysis if they contained a branch which was classed as fragmenting. In the winter experiment, this left 70 branches (35 branch pairs) split over 6 replicates. There were high levels of fragmentation in the summer experiment, therefore the results from pairs of thalli which did not fragment from 2015 and 2016 were combined into one analysis of 38 branches (19 branch pairs) split over 11 replicates.

For the field growth data, length changes per week during each time period were calculated for each thallus. Negative length changes were excluded to create a non-negative second dataset, and whether the change in each thallus during each time period was positive or negative was recorded as a third dataset for analysis. Each of these was then tested against mean temperature and global irradiance to look for significant relationships, with thallus as a random effect. To improve the normality of residuals, length change was square root transformed, and 2 extreme observations were excluded – both were negative changes, with one from August, and one from December-January. As the thalli were measured repeatedly,

an autocorrelation function (R package *nlme*, Pinheiro et al. 2015) was used to see whether there was autocorrelation between the measurements, but was not significant.

Long term distribution changes at the 46 sites were analysed using binary response variables (e.g. presence/absence, increase/no increase, stable/fluctuating). Here, substratum and time period were used as predictor variables, with a random effect of station within area, as the same station was sampled multiple times, and the stations were grouped into 3 areas (Fig. 1). When colonisation, loss, increases or decreases were examined, stations where that change was impossible were excluded (e.g. stations with absent *C. fragile* were not included in an analysis of decreases).

Results

(1) Growth of Codium fragile *under controlled conditions*

In the winter experiment, the length and weight increase of *C. fragile* after 3 weeks in the high temperature treatment (7.4 °C) was almost twice as large as the increase in the low temperature treatment (5.1 °C) (Fig. 2). After 3 weeks, mean weight gain in the low temperature was 0.3 ± 0.13 g versus 0.58 ± 0.28 g in the high temperature, and mean length gain in the low temperature was 8 ± 3 mm versus 14 ± 6 mm in the high temperature (mean ± SD, n = 35 branches in each treatment). The maximum observed individual growth over the experiment was 29 mm at 7.4 °C, and 16 mm at 5.1 °C (1.38 mm day^{-1} and 0.76 mm day^{-1} respectively). At 5.1 °C the mean RGR as calculated from weight change was 0.009 (maximum of 0.018), and at 7.4 °C it was 0.015 (maximum of 0.027). This was a statistically significant increase of around 60 % (Likelihood ratio test (LRT), $L1 = 35.0$, $P <0.001$, $R^2m = 0.28$, $R^2c = 0.67$ for weight; $L1 = 30.7$, $P <0.001$, $R^2m = 0.30$, $R^2c = 0.56$ for length). There was only one sample which showed signs of fragmentation during the experiment (here defined as length loss, weight loss over 0.2 g, or a noted observation of the thallus unravelling), and fragmentation was similarly rare in a preliminary winter experiment (results not shown). Epiphytic diatoms grew during the three weeks, but relatively slowly and did not reach high cover.

In the summer experiments, the effect of temperature on the growth was more unclear than in the winter experiment because heavy fragmentation of *C. fragile* took place. Among the thalli which did not fragment, growth tended to be higher in the high temperature treatment, but this was not as large a difference as during the winter experiment (Fig. 2). After 3 weeks, the mean weight gain in the low temperature (15-15.2 °C) was 0.75 ± 0.42 g, while it was 0.84 ± 0.68 g in the high temperature (17.9-18 °C), and the mean length gain in the low temperature was 24 ± 6 mm, while it was 28 ± 6 mm in the high temperature (mean ± SD, n

=19 branches in each treatment). RGR as calculated from these measurements was much more similar between treatments than in the winter experiment (RGR at 15-15.2 °C was 0.035 (maximum of 0.072) from length and 0.031 (maximum of 0.057) from weight, while at 17.9-18 °C it was 0.037 (maximum of 0.067) from length and 0.035 (maximum of 0.065) from weight). This was a significant difference according to length (LRT, $L1 = 8.3$, $P = 0.004$, $R^2m = 0.11$, $R^2c = 0.61$) but not according to weight (LRT, $L1 = 2.1$, $P = 0.15$, $R^2m = 0.02$, $R^2c = 0.72$). There was large variation in growth between individuals, and the maximum observed individual growth over the experiment was 37 mm at 17.9-18 °C, and 34 mm at 15-15.2 °C (1.85 mm day^{-1} and 1.7 mm day^{-1} respectively). Fragmentation occurred in both summer experiments and in a preliminary summer experiment (results not shown), and was quite evenly distributed between the temperature treatments. By the end of both experiments there was rapid growth of epiphytic diatoms.

The thalli collected in February for the winter experiment were dark green and remained so throughout. In contrast, the thalli collected in summer were bright green and fairly pale towards the tips, with a covering of fine hairs. After one to two weeks in the experimental conditions the thalli darkened in colour, and newly grown tips did not possess hairs.
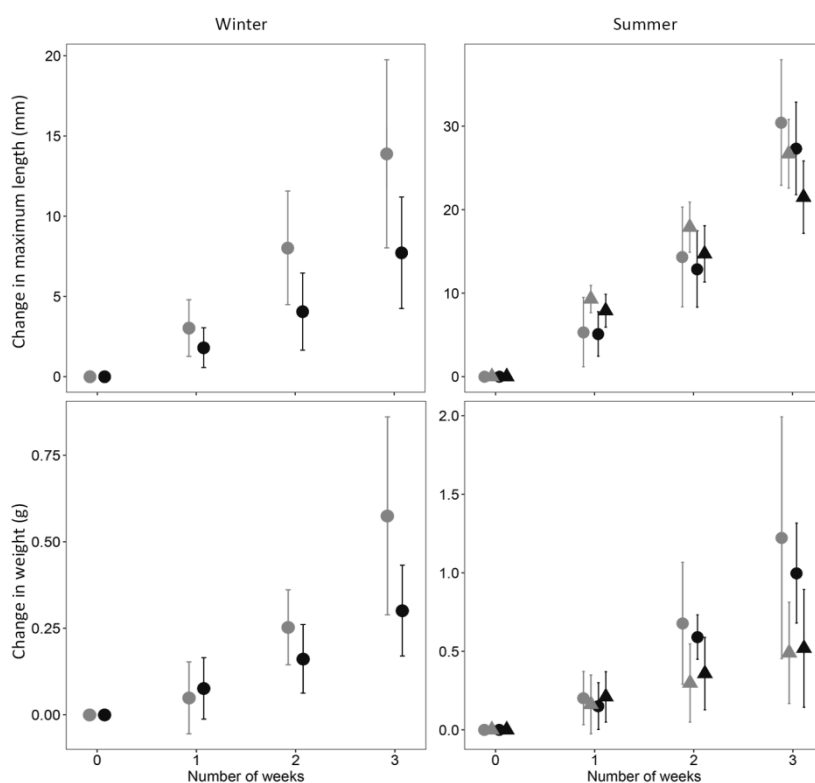

Fig. 2 Mean length and weight changes of non-fragmenting *Codium fragile* subsp. *fragile* during laboratory experiments (low temperature treatment, dark grey; high temperature treatment, light grey), with error bars showing ±1 standard deviation. In the left column are the results of the winter experiment (5.1 vs. 7.4 °C; n = 35 branches in each temperature, 6 replicates), in the right column are the results of the summer experiments (approximately 15 vs. 18 °C; n = 19 branches in each temperature, 11 replicates; 2015 as circles and 2016 as triangles). Note the different y-axis scales

(2) Growth of Codium fragile *in the field*

On average, the thalli increased in length throughout spring and summer, and decreased in autumn and winter. Length change was positively related to both temperature and irradiance (temperature, LRT, $L1$ = 12.2, *P* <0.001; irradiance (quadratic), $L1$ = 4.9, *P* = 0.028; R^2m = 0.26, R^2c = 0.26). However, a comparison of SST and mean global irradiance in Bergen showed that their minima and maxima do not occur at the same time, with temperatures lagging behind irradiance. The lowest irradiance usually occurs from late October to the end of February (<3 MJ m^{-2} day^{-1}), while the lowest temperatures occurred from January to mid-April (5 – 7 °C). Mean length change of *C. fragile* became negative during October, when irradiance drops to < 3 MJ m^{-2} day^{-1} in an average year, but temperatures were still around 11 °C; conversely, mean length change became neutral or positive again in March-April, when irradiance reaches > 4 MJ m^{-2} day^{-1} in an average year but temperatures were still < 7 °C (Figs. 3a and 3b).

The fastest growth rates were observed from late June to mid-September at around 8-10 mm per week (Figs. 3c and 3d). When only non-negative length change was considered, it showed a significant positive association to SST (LRT, $L1$ = 45.9, *P* <0.001; R^2m = 0.31, R^2c = 0.37), with the thalli showing reduced growth rates at temperatures less than 12.5 °C (Fig. 3c). Non-negative length change was not related to irradiance (LRT, $L1$ = 1.85, *P* > 0.05), remaining low from early March to mid-May, even though irradiances usually increase from around 4.6 to 13.7 MJ m^{-2} day^{-1} during this period (Fig. 3d).

Every thallus showed length reduction at some point during the year, but this was most common between late October and late February, when irradiance is usually < 4 MJ m^{-2} day^{-1} and temperatures varied from 5.8 to 11.3 °C (Figs. 3e and 3f). The proportion of thalli with negative changes decreased in March and April, and then was very low until an increase in August. The probability of a thallus showing a negative length change was negatively related to both mean temperature and irradiance (temperature, LRT, $\chi^2$1 = 7.0, *P* = 0.008; irradiance, χ^2_1 = 23.8, *P* < 0.001). Despite the frequency of negative length change in winter, many of the thalli maintained much of their length year-round, with a February mean length of 127 mm. The largest length attained was 370 mm.

Some observations on thallus appearance and length reduction were recorded during the monitoring period. In summer, the upper branches of the thalli were nearly always firm and growing. Lower down however the branches could develop small unravelling sections which could easily break, particularly in large thalli, resulting in the loss of an otherwise firm and growing branch. The branches from this "summer fragmentation" were often observed floating on the surface of the sea. In contrast, the tips of branches often (but not always)
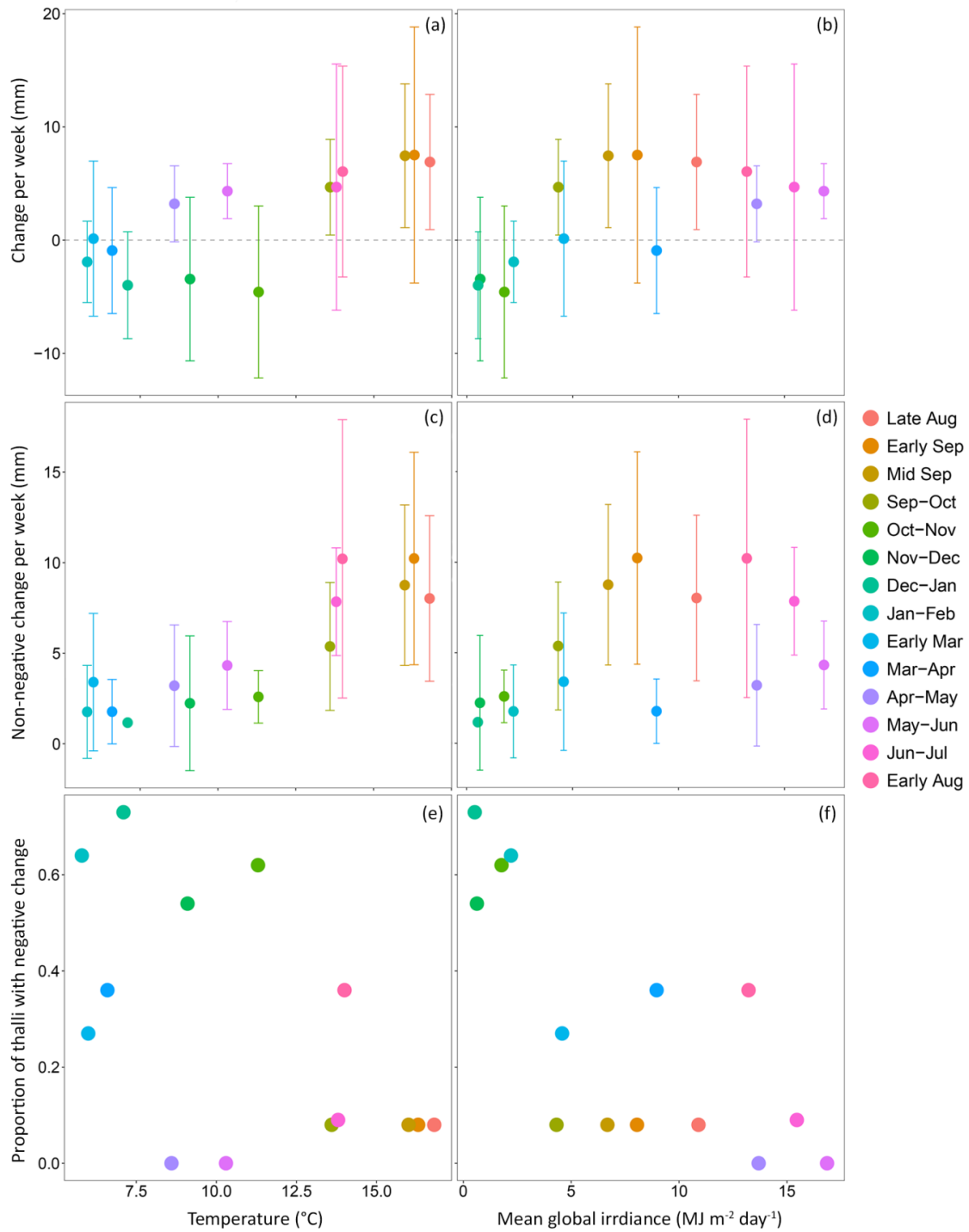
Fig. 3 Mean length changes of *Codium fragile* subsp. *fragile* thalli in the field (shallow sublittoral, Espegrend Marine Biological station, Bergen, Norway). In all plot pairs, the left hand plot shows relation to temperature (1 m depth), and the right hand plot shows relation to mean global irradiance (Bergen, GFI, 1963-2013). Plots (a) and (b) show mean change per week (here, 2 extreme values were excluded: -86 mm in July-August; -51 mm in December-January). Plots (c) and (d) show mean non-negative change per week (where all negative changes were excluded). Plots (e) and (f) show the proportion of thalli at each measurement point which had negative length changes. Error bars are ±1 standard deviation (n = 13 from August to December-January, subsequently n = 11 due to the loss of 2 thalli and their basal rocks in an unusually large storm)

became flaccid and started to disintegrate in winter, resulting in length reduction by loss of volume and unravelling from the tip. Length reduction by breakage could also occur in winter - unravelling of the thallus could occur further down the branches on some thalli, and a powerful storm resulted in sections torn from several thalli, with one of the largest thalli being reduced to a holdfast. The upper branches of many thalli also changed appearance during the year, from dark green during winter to a very bright green by June-July, especially in the larger thalli, while basal parts stayed dark green. The thalli usually remained quite epiphyte-free, despite heavy epiphytic growth on the baskets themselves during late spring and summer. There were only two relatively short periods of high epiphytic cover on *C. fragile*: diatoms in March and brown filamentous algae in April-May. Two years after placement (August 2016), most of the original *C. fragile* thalli were still present. There had also been extensive settlement of *C. fragile* on the plastic baskets and ropes, which was not there in August 2015. The size range of the new thalli was approximately 1 - 20 cm.

(3) Local distribution of Codium fragile *through time*

During the 5 years of the study (2011-2016), the average 1st quarter (Jan-Mar) and 3rd quarter (Jul-Sep) temperatures were 5.5 °C and 15.3 °C, respectively (mean of all datasets).There was one particularly cold, and one particularly hot period (Fig. 4). In 2013 temperatures dropped to 2.7 °C in Raunefjord, with a mean of 3.3 °C from the end of January to mid-March (n=5, UiB CTD dataset). The subsequent winters were milder, with no daily mean temperatures below 4.7 °C. Winter/spring in 2012 and 2015 were warm, particularly the latter with a January-March mean of 6.2 °C. The summers were around average, with the exception of 2014, when the July-September mean was 17 °C, with maximum temperatures of 21.5 °C (Fig. 4). Salinity measurements to the north of the study area (CTD dataset, University of Bergen, Fig. 1) indicate that surface salinity may fluctuate slightly but not to extremes: from 2011-2016, the mean salinity at 1 m depth was 30.0 ±1.6 (SD, n=134), with the minimum recorded being 24.4 and the maximum being 32.6, with no large seasonal differences (Jan-Mar mean = 30.9 ±1.1, Apr-Jun mean = 30.4 ±1.3, Jul-Sep mean = 28.9 ±1.3, Oct-Dec mean = 28.8 ±1.7).

The proportion of sites categorised as having high abundances of *C. fragile* remained higher among stony stations than bedrock stations (Fig. 5), with *C. fragile* significantly more likely to be present at stony stations across the whole time period (LRT, $\chi^2_1 = 17.0$, $P < 0.001$). There was also an effect of year on presence (LRT, $\chi^2_3 = 9.0$, $P = 0.030$), with a decrease in the number of stations with *C. fragile* present from 2011 to 2014, and an increase from 2014-2016 (Fig. 5). The difference between 2011 and 2014 was not statistically significant, but

there were significantly more stations with *C. fragile* present in 2016 than 2014 (pairwise comparison (Tukey), z = -2.69, *P* = 0.036).

The number of stations at which *C. fragile* decreased in abundance between surveys also showed this temporal trend (LRT, χ^2_2 = 9.73, *P* = 0.008), with decreases more likely in the 2011-2014 period than between 2014-15 (pairwise comparison (Tukey), z = 2.52, *P* = 0.031) or 2015-16 (z = 2.36 *P* = 0.047) (Fig. 6). However, there was no statistically significant trend in the increases in *C. fragile* abundance over the different time periods, and neither increases nor decreases were related to substratum type (Fig. 6).

Colonisation was not analysed statistically as there were few stony stations which *C. fragile* had not already colonised, preventing detection of any increases in colonisation rate. However, the trend for bedrock stations in Figure 7 suggests that colonisation rates were relatively constant throughout the study period. Loss could be analysed (as there were plenty of stations of both substratum types where this could occur), and the results indicated that losses were more likely at bedrock than stony stations (LRT, χ^2_1 = 5.73, *P* = 0.017) and that chance of loss was related to time period (LRT, χ^2_2 = 9.83, *P* = 0.007). Losses were significantly more likely during 2011-2014 than during 2015-16 (pairwise comparison (Tukey), z = 2.36, *P* = 0.048), while comparison of 2011-2014 to 2014-2015 only showed a trend of fewer losses in the latter period (z = 2.19, *P* = 0.073) (Fig. 7).
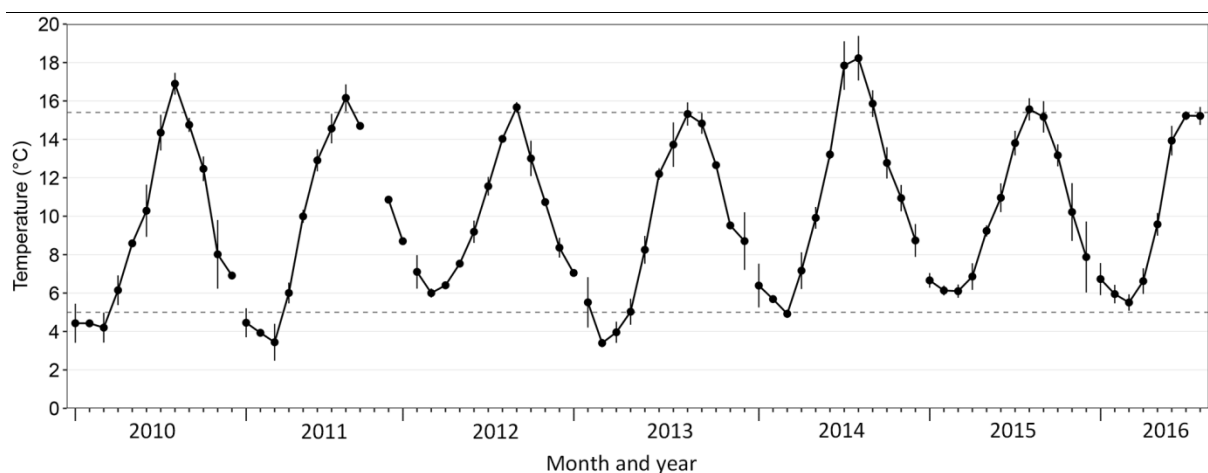

Fig. 4 Mean monthly water temperatures in the study area from January 2010 – August 2016 at approximately 1 m depth. The means (±1 SD) are calculated from the monthly means of temperature at 4 stations: a continuous logger at Espegrend (Jan 2014 - Aug 2016), a CTD dataset from the University of Bergen, and two hydrographic stations run by the Institute of Marine Research (IMR 2016) (see Fig. 1 for locations). Some of the data series had gaps present, see Electronic Supplementary Material 3. Horizontal dotted lines represent the 1980-2009 August and February means at the IMR hydrographic stations (IMR 2016)
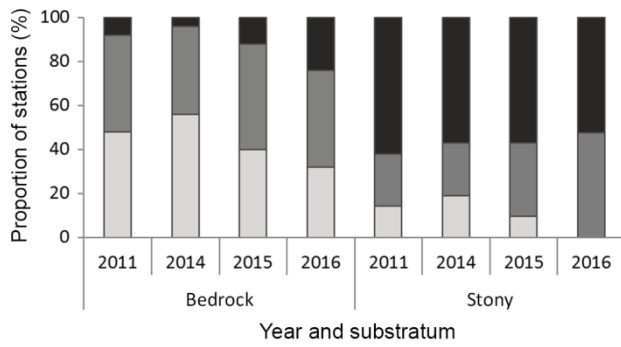
◄ **Fig. 5** *Codium fragile* subsp. *fragile* abundance at survey stations in southwestern Norway by substratum and year, with high abundance as dark grey, low abundance as medium grey, and absent as light grey (n = 25 for stations with only rock substratum ('bedrock') and n = 21 for stations with stony substratum present ('stony'))
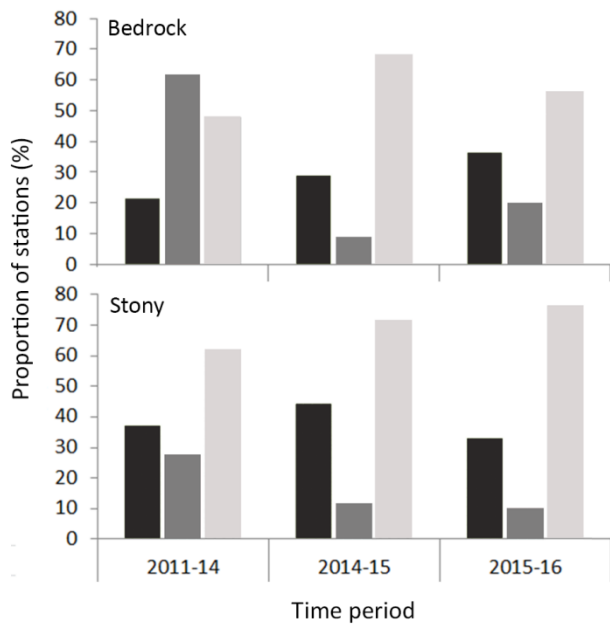
◄ **Fig. 6** Frequency of *Codium fragile* subsp. *fragile* abundance increase (dark grey), decrease (medium grey), and no change (light grey) at survey stations over time, shown as a percentage of the stations where the change could possibly occur. Results are split by substratum but no significant difference was found between substratum types (n = 25 for stations with only rock substratum ('bedrock') and n = 21 for stations with stony substratum present ('stony'))
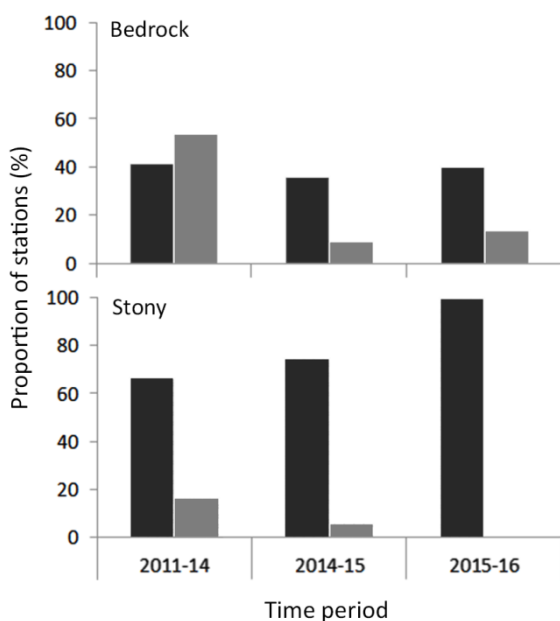
◄ **Fig. 7** Frequency of *Codium fragile* subsp. *fragile* colonisation (dark grey) and loss (medium grey) at survey stations over time, shown as a percentage of the stations where these processes could possibly occur, split by substratum (n = 25 for stations with only rock substratum ('bedrock') and n = 21 for stations with stony substratum present ('stony')). "Colonisation" and "loss" were defined by categorising the *C. fragile* abundance changes which occurred between two surveys, with changes from absence to presence at a station as "colonisation", and from presence to absence as "loss". Many stony stations already had *C. fragile* present, leaving few where colonisation could occur. Therefore the proportion colonised in the lower plot actually represents only a few stations (2011-2014, 2 of 3 potential stations colonised; 2014-2015, 3 of 4; 2015-2016, 2 of 2)

The persistence of *C. fragile* (i.e. whether it was present at the station during every survey) was clearly related to substratum type, with stony stations significantly more likely than bedrock stations to have *C. fragile* consistently present (LRT, $\chi^2_1 = 12.3$, $P < 0.001$). The majority of bedrock stations were nonetheless inhabited by *C. fragile* at some point during the study. At the stony stations, 16 of 21 stations had *C. fragile* present in every survey (with the remaining 5 having it present in at least one of the surveys); for bedrock stations on the other hand, only 4 of 25 stations had *C. fragile* present in every survey, with another 4 never having *C. fragile* present, and the rest (17 stations) having it present in at least one survey. Stability of *C. fragile* abundance (i.e. whether a station fell into the same abundance category in every survey) was also more likely at stony stations (LRT, $\chi^2_1 = 4.0$, $P = 0.045$), but in general abundances fluctuated often, with only a 48 % predicted chance of *C. fragile* abundance remaining in the same abundance category for all years at stony stations (and just 20 % for bedrock stations).

Some general observations were made when surveying the stations. On stony substratum *C. fragile* could grow in large patches, mostly within a vertical range from mean low spring water down to a few metres into the sublittoral. *Fucus serratus*, and *Ascophyllum nodosum* above that, were usually present in the littoral. Patches of *C. fragile* on stones could be dense or sparse, but normally had few other macroalgal species between (excepting *Chorda filum* in summer, and sometimes *Saccharina latissima*) (Fig. 8). At bedrock stations on the other hand, *C. fragile* mostly occurred around mean low water and only patchily or in a thin zone, vertically above kelp (*Laminaria digitata* or *Saccharina latissima*; Fig. 8). Here *C. fragile* was often growing on a "turf" formed of encrusting coralline algae colonised by various red filamentous algae (including, often, the non-native *Bonnemaisonia hamifera*; Fig. 8). Patches of this turf could be found in gaps between *Fucus serratus*, and at several sites a zone of open turf was present with absent or sparse fucoids (Fig. 8). These turf patches/zones were a common place for *C. fragile* to occupy on bedrock. At both types of sites it was relatively rare to see *C. fragile* in the littoral zone, exposed to the air at low tide.
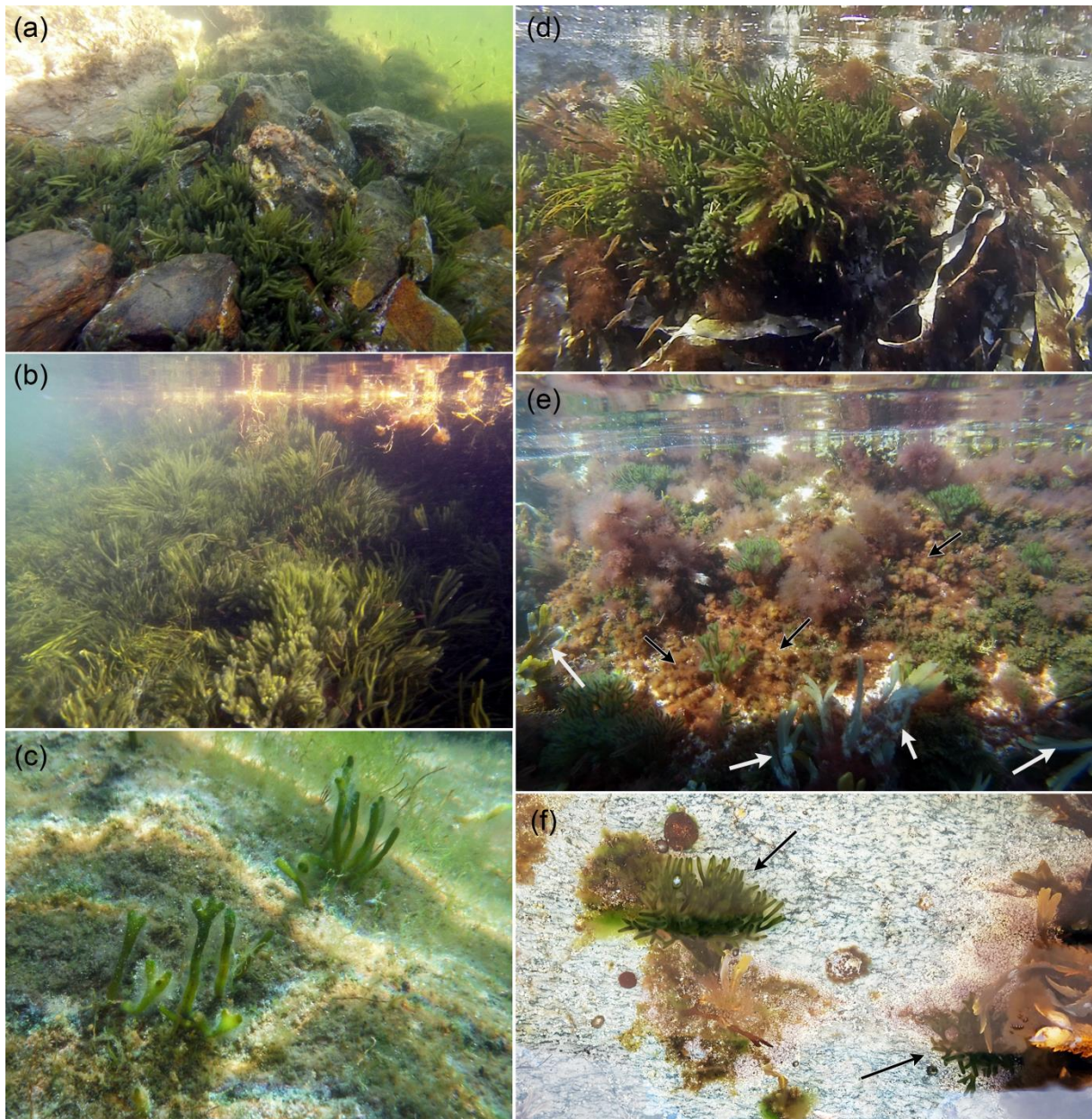
Fig. 8 The growth habit of *Codium fragile* subsp. *fragile* at some monitoring stations in southwestern Norway. Photos (a), (b), and (c) show the habit of *C. fragile* on stony substratum, where it may form sparse or dense patches stretching into the sublittoral. The majority of thalli may be between stones (a), but there may also be recruitment onto the flat surface of stones (c). Photos (d), (e) and (f) show the habit of *C. fragile* on bedrock substratum, where it tends to grow close to mean low water above the kelp zone ((d), *Laminaria* spp. in a zone below *C. fragile*), alongside or instead of fucoids such as *Fucus serratus* ((e), *F. serratus* shown by white arrows). In cases where there is a turf zone around mean low water, *C. fragile* is often present either in patches or as scattered individuals (d, e). The non-native seaweed *Bonnemaisonia hamifera* was often observed in these turf zones ((e), brown-orange tufts as indicated by black arrows). Photo (f) shows *C. fragile* (black arrows) on bedrock substratum, where it is occurring in the patches of coralline and turf algae

Discussion

Growth rate

Temperature affects the physiology and biochemistry of seaweeds (Eggert 2012), often setting the limits of their distribution range (van den Hoek 1982). Macroalgal species (and populations) genetically adapt to the temperature ranges they usually experience, but some also develop mechanisms which allow them to improve growth, photosynthesis or survival at non-optimal temperatures (acclimatisation). Ability to acclimatise is common in seaweeds, particularly among temperate species which tend to experience the largest environmental variations (Eggert 2012).

This environmental variability certainly applies to southwest Norway, where macroalgae must grow under strong, asynchronous cycles of light, temperature and nutrients. The maximum and minimum temperatures (August and February) lag behind the maximum and minimum light levels (June and December), which change rapidly during March and September due to the high latitude. Meanwhile, nutrient levels are relatively high from September to the end of March but very low in summer when more light is available (Strömgren 1986, Pedersen and Borum 1996, Armitage et al. in press) (Fig. 9). Different factors may therefore limit *C. fragile* growth at different times of the year. In the laboratory experiment, where light and nutrient levels were relatively high, *C. fragile* thalli grew at all temperatures tested (5.1-18 °C) with a positive effect of temperature on growth. However, in the field, average length changes between late October and March were not positive, despite temperatures ranging from 5-11 °C. This indicates that temperature is not the only constraint to *C. fragile* growth in nature.
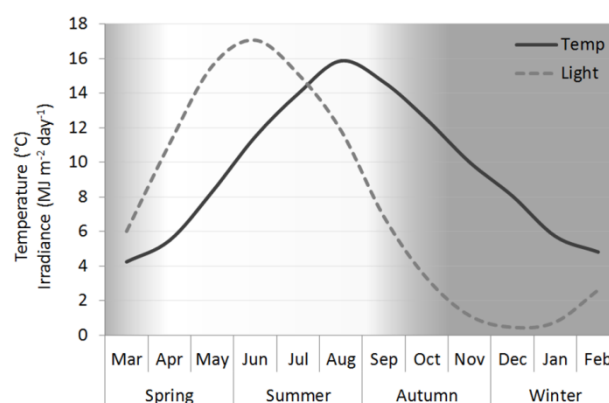

Fig. 9 Seasonal cycles of light, temperature and nutrients in southwest Norway. Temperature data from Indre Utsira hydrographic station ("Temp", 1980-2016 monthly means; IMR 2016) and global daily irradiance at the surface data from Bergen ("Light" , 1963-2013 monthly means; GFI, University of Bergen). Relative nutrient levels are shown by grey shading, where the gradient of white to grey corresponds to low to high nutrient levels (Strömgren 1986, Pedersen and Borum 1996)

In spring, when irradiance is increasing and nutrient levels are relatively high, SST is low. At this time a small increase in SST could lead to an increase in growth rate of *C. fragile*, as indicated by increase in growth rate in the winter-temperatures laboratory experiment. Summer growth rates of *C. fragile* would also be expected to show an increase in response to higher temperatures based on existing temperature studies (Hanisak 1979a), but this is uncertain in the present study given the small and inconsistent differences between 15 and 18 °C in the summer experiment, and the similar field growth rates in the summers of 2014 and 2015 (despite SST being 2-3 °C higher in the former). It may be that low ambient nutrients in the summer prevent faster growth, or that local populations are acclimatised to the present local temperature regime.

In autumn, length changes of *C. fragile* become more negative and growth slows substantially even though nutrient levels in the water are increasing and SST remains relatively high (Oct-Nov mean = 11.3 °C) (Fig. 9). At this time of year growth is probably limited by rapidly decreasing irradiance, as Strömgren (1986) has shown for several fucoids. Light saturation levels (the total daily irradiance above which there is no increase in growth with increasing light) for *C. fragile* occur at around 6 mol m^{-2} day^{-1} at 6 °C and 3 mol m^{-2} day^{-1} at ≥12 °C (Hanisak 1979a). A conversion of our global daily irradiance values to PAR (Lüning 1990) indicates values of 13.9 mol m^{-2} day^{-1} in October, 1.9-4.7 mol m^{-2} day^{-1} for November-January, 11 mol m^{-2} day^{-1} in February, and 25 mol m^{-2} day^{-1} in March. This conversion is not exact and overestimates the PAR reaching *C. fragile,* because global daily irradiance includes infra-red wavelengths and because it was measured at the land surface. Taking this overestimation into account, the values suggest that growth is unlikely to be light-limited from late February onwards, but probably are light-limited in November-January. An increase in autumn temperatures is thus not expected to have a positive effect on *C. fragile* length increases*.*

The growth rates observed at low temperatures in the present study differed somewhat from studies carried out in the NW Atlantic. Hanisak (1979a) found minimal growth after three weeks at 6 °C and no growth at <6 °C, compared to the present results of 8 mm growth at 5 °C (under the same photoperiod and irradiance). While this may have been influenced by differences in the experimental conditions (artificial vs. real seawater; 2 cm branch tips vs. larger branches; salinity of 30 vs 35), the growth rates at 18 °C were reasonably similar when compared. In addition, the observation that SSTs of 10 - 13 °C are necessary for growth of *C. fragile* in the field (Malinowski and Ramus 1973; Hanisak 1979a) does not apply here, with growing tips observed in the present study in late March-early April (6.6 °C) and mean growth rates becoming clearly positive in April-May (8.6 °C). Experiments by Malinowski (1974) showed different temperature-growth relationships between populations of *C. fragile*

(Trowbridge 1998) and, although comparison between experiments is difficult due to differences in conditions, the results of present study and of Wilson et al. (2015) are consistent with this. As *C. fragile* in the NE and NW Atlantic reproduces asexually, and is thought to be from the same genetic lineage (Provan et al. 2005), these results support the theory that acclimatisation allows *C. fragile* to become more suited to local SST regimes. However, genetic adaptation in *C. fragile* cannot be ruled out. In the experiments by Malinowski (1974), one NW Atlantic population (Boothbay Harbour) and an English population grew more than the other NW Atlantic populations of *C. fragile* at low temperatures – these populations also showed some nuclear genetic variation compared to other NW Atlantic populations (Malinowski 1974, Kusakina et al. 2006; see Benton 2014 for a discussion).

Despite a correlation between fragmentation (unravelling of the thallus, leading to length and weight reduction) and low temperatures in the field (present study, Fralick and Mathieson 1972; Hanisak 1979a), the present results demonstrate that low water temperatures (5 °C) are not sufficient or necessary to cause fragmentation of *C. fragile*, although temperature may play a role. Lack of sufficiency is demonstrated by the lack of fragmentation in the low temperature treatment in the winter experiment, while lack of necessity is demonstrated by the fragmentation observed in the summer experiment and in the field in summer. There is also considerable variation in the seasonal timing of *C. fragile* fragmentation between regions (Trowbridge 1998 and references therein), which further suggests that there are multiple factors which can cause fragmentation. Low water motion and low light levels can prevent formation of upright branching thalli, and low water motion in particular promotes dissociation of *C. fragile* thalli into filaments under laboratory conditions (Ramus 1972; Nanba et al. 2005)*.* Light may be important in southwest Norway, as there was no negative length change in the field thalli in April-June when irradiance was high, but a high frequency of negative change in late October-November when irradiance was low (despite temperature being higher in the latter). Too much water motion could also play a role, as waves may cause fragmentation of *C. fragile* through breakage (Dromgoole 1975; Bégin and Scheibling 2003). Our results showed that fragmentation is not very extensive in sublittoral sheltered environments, with many of the thalli retaining much length, which was also observed by Jorde (1966) and Trowbridge and Todd (1999). If much of the thallus is maintained year-round, this has implications for competition with winter-recruiting species.

The low levels of nutrients in surface seawater in summer in southwestern Norway (< 0.6 µmol l^{-1} nitrate, nitrite and phosphate; Armitage et al. in press) should theoretically be low enough to limit growth of *C. fragile* (Hanisak 1979b). The alga turns pale green and develops surface hairs when ambient nitrogen is low (Head and Carpenter 1975; Trowbridge 1998),

which matches our summer observations. However, the results of the present work do not support that strong limitation of growth occurs as a result. Growth rates in the summer experiment were similar to growth rates in the field, despite nutrient levels in the experiment being higher. An explanation for this which does not exclude nutrient limitation in the field is that growth in the experiment was also constrained, by epiphytic diatoms which grew in the absence of grazers. But this does not explain why the fastest field growth rates were observed in late June-August, after several months of very low ambient nutrients. How is this possible? There are indications that *C. fragile* can store some nitrogen in winter to use in spring, but this decreases rapidly, becoming relatively low in April (Hanisak 1977b). One possible explanation is that *C. fragile* has very efficient nitrogen uptake, allowing it to compete with phytoplankton for what little nitrogen is present (Hanisak and Harlin 1978). It may also acquire nitrogen through association with nitrogen-fixing bacteria (Head and Carpenter 1975). Our observations of fastest growth coinciding with low nutrient concentrations are not consistent with the experiments of Hanisak (1979a, 1979b) who found strong growth limitation of *C. fragile* at low ambient nitrogen. However, his experiments were carried out on branch tips where nitrogen-fixation rates are low (Head and Carpenter 1975) and under laboratory conditions where there is no competition for scant nitrogen.

Colonisation

Local colonisation, i.e. recruitment of *C. fragile* to stations where it was not present in the previous survey, happened relatively often, with nearly all stations colonised at least once during the 5 years. The alga has several types of propagule: vegetative large fragments, vegetative loose utricles, or swimming parthenogenetic gametes (Churchill and Moeller 1972; Scheibling and Melady 2008; Drouin et al. 2016), which may be produced at different times of year, and may be more or less effective on various substratum types. Vegetative fragments are produced whenever fragmentation takes place, but gametes are seasonal, with production and release restricted to late summer/autumn in the North Atlantic (Churchill and Moeller 1972; Hanisak 1979a). Theoretically, detached fragments of *C. fragile* could become trapped between stones, providing stability for attachment and regrowth (as may occur when fragments become trapped in littoral pools or between eelgrass rhizomes; Scheibling and Melady 2008; Gagnon et al. 2014). However, this would have little chance of occurring on sloping bedrock at tidal level with nothing to hold the fragment in place, thus smaller propagules such as gametes may be necessary for colonisation in these habitats.

The unusually hot summer of 2014 (Fig. 4) was expected to be favourable for *C. fragile* as temperatures were closer to its optimal growth and germination temperatures (Hanisak 1979a). However, there was no obvious effect on colonisation or abundance increases in the

present study. This may be because SST is already adequate for recruitment in an average year. Gamete production occurs at 12-15 °C (Churchill and Moeller 1972), which occurs during an average summer in this region (Fig. 4). And although germlings and undifferentiated filaments (during the early vaucherioid growth stage) of *C. fragile* are more senstitive to temperature than the adult thallus, temperatures in the study area should remain suitable for growth through October (Hanisak 1979a). Settlement, growth and survival of small stages must be able to occur in average summer and winter temperatures because many recruits were observed on the baskets containing the field thalli in August 2016, which were likely recruited in summer 2015/2016 given their size range (Ramus 1972). This study therefore gives no indication that hot summers will have a direct positive effect on colonisation. However, a longer term study might detect any indirect positive effects of hot summers, for example due to a reduction in native competitor species (Levin et al. 2002).

Observations of distribution patterns on bedrock substratum raised some questions for further study. *Codium fragile* was often growing where there were zones or patches of encrusting coralline and filamentous/turf algae. In the study area *C. fragile* can also be found growing on *Corallina officinalis*. Observations of *C. fragile* growing on *Corallina officinalis* and encrusting coralline algae have also been reported in SW Norway (Jorde 1966), S England (Chapman 1999), Nova Scotia (Chapman 1999, Schmidt and Scheibling 2005), and New Zealand (Trowbridge 1995). Scheibling and Melady (2008) also found that turfs of *Corallina officinalis* enhanced attachment of small lateral *C. fragile* branches in static water, although they did not see a difference in attachment strength. It is possible that the occurrence of *C. fragile* on bedrock may be related to where encrusting organisms have colonised the surface. The recruits on the plastic baskets containing the field thalli were consistent with this, as although mature gametes were observed on thalli at the site in August 2014, no recruits appeared on the baskets until August 2016, after the baskets were beginning to be covered in encrusting biota in summer 2015. The frequent occurrence of *Bonnemaisonia hamifera* with *C. fragile* in coralline patches on bedrock with sparse fucoid cover could also be interesting to investigate in the context of invader-synergy, given the allelopathic and anti-grazing effects of *B. hamifera* (Enge et al. 2013; Svensson et al. 2013) and the fact that *C. fragile* tends to establish in spaces free of other canopy species (Levin et al. 2002; Scheibling and Gagnon 2006).

Persistence

Persistence of *C. fragile* was high at stony stations, while loss was more frequent at bedrock stations, particularly in the period with the cold winter when it was lost from around 50 % of rock stations. The periods with mild winters also had significantly fewer decreases in *C.*

fragile abundance than the period with a cold winter. The low temperatures during that period (2.7 °C) are not low enough to cause mortality of *C. fragile* according to experiments (Lüning 1984) and a direct effect of temperature on survival would not explain the influence of substratum, since losses were more common on bedrock even in mild years. Instead, the results suggest that *C. fragile* is more prone to death or detachment at bedrock stations, and that low temperatures exacerbate the effect.

One potential explanation for this is physical water forces. Piles of stones provide micro-habitats with low water motion, protecting *C. fragile* holdfasts which lie between them. These remains or trapped fragments can then regrow even if extensive fragmentation occurs. A positive effect of this kind occurs when mussels grow around the base of *C. fragile*, improving its survival by protecting the holdfast from waves (Bulleri and Airoldi 2005). In contrast, water motion may pull *C. fragile* free on bedrock substratum (D'Amours and Scheibling 2007). In addition, *C. fragile* usually grows near tidal level on bedrock, and therefore is more exposed to wave impact. Waves may lead to cumulative damage to the attachment of *C. fragile* through twisting and pulling (D'Amours and Scheibling 2007), potentially making loss more likely in the event of strong waves. A cold winter could enhance this effect if it reduced attachment strength further.

Other possible explanations include competition and grazing. Competitive interactions with kelp can reduce *C. fragile* growth (Scheibling and Gagnon 2007) and intact kelp canopies are thought to inhibit *C. fragile* recruitment (Levin et al. 2002). However, these effects could vary by substratum, as the lower stability of stony substratum compared to bedrock may influence the distribution of large canopy algae. Larger thalli are expected to experience higher drag forces, which may increase dislodgement chances of a stone they are attached to if it is loose (Sousa 1985). The effect of grazing may also vary by substratum, as gaps between stones can provide refuge from larger grazers (Lubchenco 1980; Scheibling et al. 2008), allowing fragments of the thallus to remain. Field experiments have suggested that littoral grazers such as *Patella vulgata* do not exert much control on *C. fragile* (Trowbridge 1995; Trowbridge et al. 2016), but grazing by *Littorina littorea* can reduce survival of small or damaged individuals (Scheibling et al. 2008). These would be the grazers closest to *C. fragile* on bedrock where it grew around mean low water, while in the sublittoral, where *C. fragile* often occurs on stony substratum, urchins may be present (*Echinus esculentus* and *E. acutus*). While the grazing habits of *Echinus* spp. on *C. fragile* are unknown, the urchin *Strongylocentrotus droebachiensis* prefers kelp (although also eats *C. fragile*), and at low densities will bypass *C. fragile* to graze on kelps nearby (Scheibling and Anthony 2001; Lyons and Scheibling 2008). *Codium fragile* produces dimethylsulfoniopropionate (DMSP) which can make it unpalatable to some grazers (Lyons et al. 2007). However, some

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sacoglossan sea slugs prefer and actively feed on *C. fragile* (Trowbridge 2004). Establishing how grazers affect *C. fragile* distribution, and how this might interact with substratum and temperature, is therefore complex, with grazing effects varying by grazer species, by status of the *C. fragile* thalli, and by which other native seaweeds are available.

Local and vertical distribution

Different types of hard substratum are not often discussed in studies of *C. fragile*, but should be considered in future work. The present distribution of *C. fragile* in this area is strongly associated to this factor, with persistent, sometimes large and dominant, infralittoral and sublittoral populations on stones, and intermittent and fluctuating infralittoral populations on bedrock. The results suggest that these patterns occur because *C. fragile* populations on bedrock are vulnerable to loss, rather than due to a lack of colonisation, but it requires further experiments to establish whether this is the case. If so, it resembles a source-sink dynamic (Pulliam 1988), in which populations persist on stones and provide propagules to colonise rocky substratum, where populations may expand but are likely to be lost. Although some of the relationships presented in the present study are correlative, they suggest that minimum SST plays a role in this, and that higher SST during winter and spring would increase growth, abundances and occupancy of *C. fragile*. These future effects could apply to a large habitat area on this coastline – the outer fjords and islands provide huge areas of hard substratum protected from the open sea, with salinity values well within the tolerances of C. fragile (Hanisak 1977a).

Although the focus of the distribution survey was on *C. fragile* abundances, it is possible that higher temperatures could also influence its vertical range. In the British Isles, *C. fragile* may be found on emergent substrata in the littoral zone (Burrows 1991; Chapman 1999; Trowbridge and Todd 1999), but this is not very common in southwestern Norway, even though *C. fragile* may grow in rock pools. Current air temperatures reach minus degrees Celsius in January and February nearly every year (Meteorologisk Institutt 2015), which may limit survival in the littoral (Trowbridge 1998), particularly if combined with low salinity from the high rainfall along this coast. Milder winters might therefore improve conditions for *C. fragile* in the littoral in northern areas of the Atlantic.

While kelps and other large canopy species can negatively affect *C. fragile* by overgrowth or shading, *C. fragile* can inhibit their recruitment once it is established (Levin et al. 2002, Scheibling and Gagnon 2006). Competitive interactions may also occur between *C. fragile* and the low littoral canopy-alga *Fucus serratus* (Armitage et al. 2014). Temperature changes can alter competitive relationships between canopy-forming macroalgae (Armitage et al. in

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press), and may affect species which grow in the same zones as *C. fragile,* potentially influencing these interactions. *Fucus serratus* is fairly resistant to hot summers in southwestern Norway (Armitage et al. in press), but they may cause increased blade erosion and reduced abundance of the kelp *Saccharina latissima* (Moy and Christie 2012; Armitage et al. in press) and maintain temperatures which damage and weaken *Laminaria digitata* blades (Simonson et al. 2015). Negative effects of temperature on these kelps will probably be strongest where water warms rapidly: in shallow water at non-exposed sites, places which are already favoured by *C. fragile.* It is therefore possible that the positive effects of a temperature increase on *C. fragile* may be enhanced by a negative effect on native canopy seaweeds which inhibit its establishment (Levin et al. 2002).

Positive effects of temperature on *C. fragile* may also result in additional negative effects on native algae. Currently, the abundance of *C. fragile* within a site often fluctuates and can do so greatly (Jorde 1966, present study), therefore it may not be very effective in competitively excluding native species over long time scales (Watanabe et al. 2010). Maintenance of thalli throughout winter suggests that *C. fragile* can already occupy some space year-round at sheltered sites, but if higher temperatures allow populations to become more persistent then *C. fragile* may occupy space for longer, even on bedrock substratum. This could enhance inhibition of the reestablishment of native species by *C. fragile* (Levin et al. 2002; Scheibling and Gagnon 2006).

Compliance with Ethical Standards

Caroline S. Armitage declares that she has no conflict of interest. Kjersti Sjøtun declares that she has no conflict of interest. Ethical approval: This article does not contain any studies with animals performed by any of the authors.

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