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Effects of Increasing Temperature and Ocean Acidification on the Microstages of two Populations of Saccharina latissima in the Northwest Atlantic

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Effects of Increasing Temperature and Ocean Acidification on the Microstages of two Populations of *Saccharina latissima* in the Northwest Atlantic

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Effects of Increasing Temperature and Ocean Acidification on the Microstages of two Populations of Saccharina Latissima in the Northwest Atlantic

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Abstract

Saccharina latissima (Linnaeus) C.E.Lane, C.Mayes, L.D. Druehl and G.W.Saunders, is the most widely distributed species of kelp in the western North Atlantic, occurring from the Arctic to Long Island Sound. The effects of global climate change on these ecologically and economically important cold temperate species at the southern range of their distribution are unknown. This study investigated the impact of the combined stressors of increased temperature (16, 19, 22, 25 & 28°C) and reduced pH (7.9, 7.8, 7.7, & 7.6) on the gametophyte and juvenile sporophyte stages of sugar kelp populations from Maine and Long Island Sound. Spore germination and growth, male and female ratio, fecundity, reproductive success of female gametophytes, and growth of juvenile sporophytes were investigated on crossed gradient temperature tables with CO₂-adjusted pH levels. The upper critical thermal limit for gametophytes in all trials for both populations was 22°C, with full mortality of gametophytes occurring at all temperatures tested above this limit (i.e. 25° and 28°C). Gametophyte survival, growth, and male and female ratios were similar in all trials for both populations at 16° and 19°C, but gametogenesis was suppressed at temperatures above ca. 17°C. There were no consistent effects of pH in any trials, though the lower pH values (7.6-7.7) did result in slightly larger gametophytes (primary cell diameter & gametophyte length) than the highest value (7.9) at 16° and 19°C in some of the trials. These results support the hypothesis that the predicted increase in seawater temperatures will shift the distributional boundary of these cold temperate seaweeds northward, resulting in the loss of populations at the southernmost boundary.
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Introduction

Biology and Ecology of *Saccharina latissima*

*Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, L.D. Druehl and G.W.Saunders (Lane et al., 2006) belongs to a larger group of the large brown cold temperate kelps, which are the most ecologically and economically important seaweeds in the Northern Hemisphere (Lüning et al., 1990; Yarish & Pereira, 2008). *Saccharina* species are major components of underwater ecosystems in the North Atlantic, forming extensive, highly productive beds (Mann, 1973; Egan & Yarish, 1990) that can correspond to terrestrial forests in terms of physical structure and habitat generation (Graham, 2004; Mann, 1972). The three dimensional character of kelp forests provide substrate, shelter, shade, and oxygen for other organisms (Steneck et al., 2002), including commercially important fish and invertebrates (Bologna & Steneck, 1993; Egan & Yarish, 1990; Lazzari & Stone, 2006) and contribute significantly to coastal primary production and local food webs through release of detritus, dissolved carbon, and meiospores (Graham, 2004; Duggens et al., 1989; Van Patten & Yarish, 1993).

Kelps are cultivated and wild harvested worldwide for food, medicinal, agricultural, and industrial products. Kelp can be cultured as the bioextractive component of integrated multi-trophic aquaculture systems (Yarish & Pereira, 2008; Chopin et al., 2008), and could represent a possible carbon-neutral source of biomass for fuel alternatives. Development of global kelp culture systems could be part of a mitigation strategy for problems of excess atmospheric CO$_2$ and ocean eutrophication, while providing a source of energy, food, and biochemical products for the demands of a rapidly growing global human population (Turan & Neori, 2010; Holdt & Kraan, 2011).

The family Laminariaceae, order Laminariales, is widely distributed with great morphological and physiological plasticity, lending itself to taxonomic confusion. Sporophytes of
the Laminariaceae consist of a branched holdfast, simple stipe, and smooth lamina, which can be either simple or digitate. The simple lamina group in the Northwest Atlantic includes two morphologically similar species, *Saccharina latissima* (formerly *Laminaria saccharina*), and *Saccharina longicruris* (formerly *Laminaria longicruris*) (Lane et al., 2006). These both exhibit high levels of morphological plasticity controlled mainly by environmental factors (Gerard & Mann, 1979; Fowler-Walker et al., 2006), with some level of genetic control (Chapman, 1974; Lüning et al., 1978; Yarish et al., 1990; Egan et al., 1990). These two species are interfertile (Lüning et al., 1978; Chapman, 1974), and exhibit no phylogenetic evidence of genetic difference (Neefus et al., 1993; McDevit & Saunders, 2010), While *Saccharina longicruris* is still a recognized species name (Lane et al., 2006; Guiry & Guiry, 2013), it is treated here as conspecific with *Saccharina latissima*, with the understanding that distinct genetic strains or races probably exist throughout the North Atlantic as part of a large, very plastic species complex (Bartsch et al., 2008; Cho et al., 2000; Chapman, 1974; Kain, 1979; Egan et al., 1990; Lüning, 1975).

*Saccharina latissima* has a heteromorphic life cycle that consists of an alternation of a macroscopic diploid sporophyte stage with a microscopic haploid dioecious oogamous gametophyte stage (fig. 1). Sporophytes can develop sorus tissue and release spores throughout the year (Parke, 1948; Chapman, 1984), with peak reproductive seasons occurring in the spring and fall (Egan & Yarish, 1990; Van Patten & Yarish, 1993). Haploid, flagellated meiospores (~5 µm) are released from unilocular sporangia in an equal ratio of males and females (Schreiber, 1930). Once attached to a substrate, meiospores shed flagella, germinate, and move spore cell contents into the primary cell (Lüning, 1980). After a resting stage that can be a few days to several weeks, the primary cell increases in diameter to form the “dumbbell stage”, at which point it could potentially enter gametogenesis as a male or female unicellular gametophyte, or vegetatively divide to form multicellular filaments (Harries 1932; Lüning, 1980).
Female gametogenesis is controlled by interacting environmental variables, including temperature (Kain, 1964; Lüning, 1980), light (Lüning & Dring, 1979) and availability and concentration of nutrients (Hsiao & Druehl, 1973; Dayton, 1985), and can be delayed indefinitely in the vegetative state (Druehl et al., 2005), existing as unicellular females (Lüning, 1980) or extensive multicellular filaments. After induction of gametogenesis, a single egg is extruded from mature female oogonia along with the hormone lamoxirene, which signals release and attraction of the flagellated spermatozoids from nearby males (Lüning & Muller, 1978; Marner et al., 1984). The diploid zygote immediately begins to divide longitudinally to develop into the juvenile sporophyte.

Sporophytes exhibit a seasonal growth pattern, with a general increase in growth rates in the late fall to winter and a rapid increase from late winter to spring as temperatures and light...
levels increase (Kain, 1979; Chapman & Craigie, 1977). Sporophytes have a broad growth optimum (10-15°C), a characteristic shared by other seaweed species located at the southern boundaries of cold temperate zones (Fortes & Lüning, 1980). Growth declines throughout summer and early fall with the increase in water temperature and decrease in nutrient availability. Severe blade erosion occurs at 16°C and above in Long Island Sound (Brinkhuis et al., 1983; Egan & Yarish, 1990) with high rates of mortality occurring at 20°C and above (Chapman & Craigie, 1977).

The alternation of generations in the life cycle may be a means for populations to survive unfavorable or stressful environmental conditions. The microscopic gametophyte stage is considered a ‘survival stage’, a perpetuation phase exhibiting ecophysiological differences from the large sporophyte stage (Carney & Edwards, 2006; Ladah et al., 1999). Microscopic gametophytes and juvenile sporophytes are physiologically better adapted than the sporophyte stage to withstand adverse environmental conditions including pollution (Hopkins & Kain, 1978), long periods of darkness (Lüning, 1980; Kain, 1964), and higher temperatures, up to 1-7°C (Ladah & Zertuche, 2007; Kain, 1969; Bolton & Lüning, 1982; tom Dieck, 1993). While gametophytes may be able to withstand higher summer temperatures than sporophytes, gametogenesis is inhibited at higher summer temperatures (Lüning, 1980; Egan et al., 1989); possibly creating a vegetative “resting stage” (Chapman, 1964) that might allow populations at the southern limit to persist through periods of adverse temperatures. Gametophytes may be able to exist in a dormant vegetative state indefinitely (Dayton, 1985), surviving up to 30 years in the lab under vegetative conditions while still retaining sexual capacity (Neushul, 1987).

*Saccharina latissima* is a widely distributed species in the Northwest Atlantic, ranging from the Arctic in the North to around the 20°C August seawater isotherm in the south (van den Hoek, 1982; Egan & Yarish, 1988), which stretches across the Atlantic from Long Island Sound to the Iberian Peninsula. The Gulf of Maine represents the middle latitudinal range of the distribution, experiencing lower summer temperatures than Long Island Sound, occurring
approximately where the 15°C summer isotherm stretches across the Atlantic to England (Lüning, 1990). The distribution range of *Saccharina latissima* is unique among other northern macroalgal species, in that it extends further south than Cape Cod, a major phytogeographic boundary on the East Coast of the U.S. (Mathieson, 1979).

Widely distributed species experience different temperature and light regimes throughout the year, and this can create distinct differences among populations (Lüning & Neushul, 1978). The large-scale temporal variations in temperature, light (including irradiance and photoperiod), and nutrient availability (Dayton et al., 1999) are more important in determining kelp demography than small-scale local processes of competition and grazing (Rinde & Sjotun, 2005). Latitudinal variation can influence seasonal growth and life history patterns, upper lethal temperatures, and reproduction, and these differences can be seen in *Saccharina latissima* populations from the southern and middle ranges of their distribution. The cooler waters of the more northerly middle latitudes typically allow for a longer growing season for sporophytes (January-July), and plants can persist through summer temperatures, where they can be perennial up to 3 years of age (Parke, 1948; Kain, 1979; Johnston et al., 1977). Reproductive sorus tissue forms on blades that are at least six months of age in the mid-range, and new spring juvenile sporophytes have higher rates of attachment and survival than new winter plants (Parke, 1948).

Southern range populations experience a shorter growing season (January-May), with plants disintegrating or disappearing in late summer due to high water temperatures (Brinkhuis et al., 1983; Lee & Brinkhuis, 1986) and low levels of nitrogen (Egan and Yarish, 1990). The majority of sporophytes are annuals, but a percentage of the population are biennials (Egan &Yarish, 1990), recovering from severe blade erosion and continuing growth the following fall if enough of the meristematic tissue survives (Lee & Brinkhuis, 1988; Wong, 1989; Brady-Campbell et al., 1984). The frequency of annuals increases south of Cape Cod, suggesting more extreme environmental conditions (Coleman & Mathieson, 1975; Sears & Wilce, 1975).
Reproductive sorus tissue can develop earlier, on tissue that is only four months old and as small as 15 cm in length. New spring plants in Long Island Sound rarely survive through the summer, while new winter plants have higher survival rates and can persist into the following year (Parke, 1948). Brinkhuis et al. (1984) suggested that the late spring sporogenesis period was an adaptation to the higher temperatures in Long Island Sound, since other mid and northern range populations had been observed to release spores mainly in winter (Kain, 1979).

Seaweeds exposed to climactic stressors (e.g. glaciation cycles) and unique local conditions (e.g. temperature, nutrient levels) are subject to strong selection pressure and local adaptation. Periods of isolation and gene transfer of *Saccharina latissima* populations have resulted in a genetically diverse species across the northern oceans, with distinct and hybridized clusters found in the Eastern and Western Atlantic, Arctic, and Eastern Pacific (McDevit & Saunders, 2010). Regionally, populations have adapted to different environmental conditions (Lüning & Neushul, 1978), including different light regimes (Gerard, 1988; 1990), nitrogen supply (Gerard, 1997; Espinoza & Chapman, 1983), and temperature (Gerard & Du Bois, 1988). The differential responses of gametophytes and sporophytes between middle range and southern range *Saccharina latissima* populations in the Northwest Atlantic have been attributed to ecotypic differentiation (Russell, 1986, Breeman & Pakker, 1994).

**Effects of Temperature on Saccharina latissima**

Seaweeds with heteromorphic life stages often experience seasonal shifts in optimal environmental conditions that can vary with development and age (Lüning, 1984; Lobban & Harrison, 1997; Fortes & Lüning, 1980). Microscopic stages of *Saccharina latissima* cultivated at different times of the year have exhibited different light intensity and temperature requirements for optimal growth (Brinkuis et al., 1984; Egan et al., 1989). Shifts in temperature optima and maximum survival temperatures have been observed in southern-range populations, indicating acclimation to warmer summer temperatures (Lee & Brinkhus, 1988; Egan et al., 1989; Kain,
Sporophytes of *S. latissima* can tolerate temperatures up to 18°C, with seasonal shifts of up to 5°C (Lüning, 1984), and similar shifts have been observed in gametophyte temperature tolerance in summer months (Egan *et al.*, 1989; Lee & Brinkhuis, 1988). Laminarian gametophytes have exhibited upper tolerance temperatures 1-7°C above tolerance limits for sporophytes (tom Dieck, 1993).

Several studies have observed distinct ecotypical differences in physiological response to environmental variables of light, temperature, and nutrients between populations from the Gulf of Maine and Long Island Sound. Sporophytes collected from Maine exhibited negative growth rates at 20°C after two weeks, and did not survive three weeks at this temperature, while individuals collected from Long Island Sound survived and experienced positive growth rates for up to 6 weeks at 20°C (Gerard & Du Bois, 1988). Sporophytes from Long Island Sound also exhibited better growth rates under low light conditions (Gerard, 1990), which might be a result of the increase in the major light harvesting pigments (chlorophyll a, chlorophyll c, and fucoxanthin) that occurs under higher temperature conditions (Davison *et al.*, 1991). These plants also accumulated more tissue nitrogen than sporophytes from Maine, supporting resilience of the photosynthetic apparatus and possibly contributing to heat shock proteins, which are important in high temperature stress survival (Gerard, 1997). Other studies have also observed increased survival of kelps in the presence of adequate nutrients (Dayton, 1985), including a study of a southern-limit population of *Saccharina japonica* in China that was found to survive summer temperatures up to 25°C in the presence of increased levels of nitrogen, presumably contributing to formation of heat stress proteins and strengthening of the photosynthetic machinery (Liu & Pang, 2010).

Long Island Sound gametophytes and juvenile sporophytes have exhibited higher temperature tolerance and more sustained positive growth than individuals obtained from northern populations (Egan *et al.*, 1990) (Table 5). While the lethal limit for meiospores is 22°C for populations from higher latitudes (tom Dieck, 1993), meiospore germination has been
successful at temperatures as high as 25°C in July and October in one study (Egan et al., 1989), but not in other months. Gametophytes from Long Island Sound have exhibited a shift in optimal growth temperature from 10-15°C in March to 15-20°C in July, and successfully produced young sporophytes at 20°C in all months except January (Egan et al., 1989). Gametophytes from the middle range also have a broad optimal range for growth, from 10-15°C (Bolton & Lüning, 1982), to 10-19°C (Lüning, 1980), but have lower temperature limits for gametogenesis, producing sporophytes at temperatures below 16°C (Harries, 1932), or 18°C (Evans, 1965; Bolton & Lüning, 1982; Lüning, 1984). Lethal temperatures for Long Island Sound gametophyte and juvenile sporophyte populations have also been reported to be higher than mid-range populations, at 25°C (Egan et al., 1989), compared to reported values of 22°C (Lüning, 1980, 1984: Kain, 1969) and 23-24°C (Bolton & Lüning, 1982: Breeman, 1988). Juvenile sporophytes from the mid-range exhibited optimal growth at 10-15°C, reduced growth at 20°C, and disintegration of plants at 23°C (Bolton and Lüning, 1982).

**Ocean Climate Change**

Anthropogenic alterations of the carbon cycle through fossil fuel combustion and changes in land use have resulted in the rapid increase of atmospheric CO₂ from pre-industrial levels, with predictions of a 2-3 fold increase of current levels by year 2100 (IPCC, 2007, 2013), and a 5-fold increase by year 2300 (IPCC, 2013, Caldeira & Wickett, 2003). Increased levels of CO₂ along with other greenhouse gases, including methane and nitrous oxide, have resulted in a warming of the global mean surface air and ocean temperatures (Levitus et al., 2000), with a predicted increase of overall ocean temperatures of about 0.2°C or more per decade (IPCC, 2007, 2013). Global scale temperature increases are expected to impact coastal ecosystems through sea level rise, increased storm events, changes in hydrologic cycles, and changes in ocean circulation (IPCC, 2007, 2013; Scavia et al., 2002).
The ocean acts as a major inorganic carbon reservoir in the global carbon cycle; slowing the accumulation of atmospheric CO$_2$ by absorbing ca. 30-50% of all anthropogenically released CO$_2$ (Sabine et al., 2004; Doney et al., 2009). Atmospheric CO$_2$ dissolves into seawater directly or reacts with water (H$_2$O) to form carbonic acid (H$_2$CO$_3$). Carbonic acid is a weak acid that readily disassociates into hydrogen (H$^+$) and bicarbonate (HCO$_3^-$) ions. Carbonate (CO$_3^{2-}$) ions act to buffer this increase in hydrogen ions by reacting with some of the available H$^+$ ions to form more bicarbonate (HCO$_3^-$). The increased absorption of CO$_2$ from the atmosphere results in a net increase of carbonic acid, free hydrogen, and bicarbonate ions, but a decrease in overall carbonate ion concentration. This shift of the carbonate chemistry of seawater decreases seawater pH and reduces the buffering capacity of seawater (Sabine et al., 2004). Overall surface pH levels are predicted to decrease between 0.14 and 0.35 units during this century (IPCC, 2007, 2013), with a maximum pH reduction at the ocean’s surface of 0.77 units at levels near 2000ppm, predicted by year 2300, with potential profound ecological shifts in marine ecosystems (Doney et al., 2009).

The steep changes in atmospheric CO$_2$ and global temperatures over relatively short time scales are expected to have extensive effects on the physical and biological dynamics of the world’s oceans. These changes could impact ocean temperature, weather patterns, biogeochemistry, salinity levels, sea level, UV radiation, current circulation patterns, stratification, upwelling, ocean chemistry, and oxygen levels. Increased ocean temperature and acidification can affect all marine species’ physiology, including reproduction, growth and survivorship. The biological and physical changes can act together to affect marine ecological communities by impacting species distribution, abundance, biogeography, and species interactions (Rilov & Treves, 2010).

It is unclear how a shift in carbonate chemistry will affect non-calcifying photosynthetic organisms like kelp, but the increase in carbon dioxide and bicarbonate is likely to increase the concentration of carbon available for photosynthesis. Most marine autotrophs are characterized
by C3 carbon fixation (Koch et al., 2013), which allows organisms to utilize inorganic carbon from both dissolved carbon dioxide (CO$_2$) gas and the much more abundant bicarbonate (HCO$_3^-$) ions. Bicarbonate ions are utilized through a set delivery of inorganic carbon to the Rubisco enzyme by a carbon concentrating mechanism (CCM) (Lucas, 1983; Badger & Price, 1994). Carbon concentrating mechanisms work with carbonic anhydrase to form CO$_2$ from HCO$_3^-$ through a dehydration reaction, allowing algae to draw from a much larger carbon pool for photosynthesis.

Both gametophytes and sporophytes of kelp species have detectable intracellular and extracellular carbonic anhydrase activity, and both are capable of utilizing bicarbonate, though sporophytes have a greater capacity for HCO$_3^-$ use than gametophytes (Zhang et al., 2006). External carbonic anhydrase activity allows Saccharina latissima to maintain photosynthetic rates over a wide range of pH (7.0-9.5) (Axelsson et al., 2000), though rates begin to decline at high pH, at values above 8.5 (Blinks, 1963). While CCM’s allow most macroalgae to utilize the greater HCO$_3^-$ carbon pool, an increase in availability of the preferred CO$_2$ is expected to somewhat increase photosynthetic and growth rates (Koch et al., 2013).

While the increase in available and preferred dissolved CO$_2$ could enhance photosynthetic rates, the accompanying change in pH could affect macroalgae in other ways. Effects of reduced seawater pH on macroalgae have so far been variable; with minimal to no differences as well as positive and negative responses (Israel & Hophy, 2002). In areas of natural acidification near underwater vents, large brown macroalgae (Ochrophyta) remain the dominant canopy forming species across a CO$_2$ gradient with a pH range from 8.20-6.07 (Porzio et al., 2011). In laboratory studies, very high levels of CO$_2$ (3000ppm) inhibited growth of the sporophytes of Saccharina latissima, but enhanced growth of kelp species Nereocystis luetkeana (Swanson & Fox, 2007). There appears to be a difference in response according to the level of dissolved inorganic carbon (DIC) present, where net apparent productivity of N. luetkeana sporophytes was enhanced at two times ambient CO$_2$, but reduced at five times the
ambient levels (Thom, 1996). The red seaweed *Lomentaria articulata* responded in a similar way, with increased growth at two times ambient levels, but not at five times (Kubler *et al.*, 1999). *Macrocystis pyrifera* (giant kelp) gametophytes were 32% larger at moderate (7.86) pH, but only 10% larger at low pH levels (7.61) (Roleda *et al.*, 2012). High levels of dissolved inorganic carbon (1200 ppm) and resultant low seawater pH (7.61) had minimal effects on meiospore germination rate, gametophyte sex ratio and size of *M. pyrifera* gametophytes, though very high levels decreased spore germination by 6-9% (Roleda *et al.*, 2012).

Kelp population structure and dynamics are determined by successful life stages, which include spore output, dispersal, recruitment of meiospores, development and survival of gametophytes, successful reproduction, and development and survival of the juvenile sporophytes. These stages are primarily controlled by environmental factors including temperature, light, photoperiod, and nutrient availability, all of which could be affected by a changing ocean. Very little is known about species-specific responses to interacting variables of increasing temperature and CO₂ (Koch *et al.*, 2013). An increase of CO₂ can offset high temperature stress in some terrestrial C3 plants, but increased temperatures may reduce availability of dissolved CO₂ in seawater, due to a decrease in solubility (Beardall *et al.*, 1998). Little is known about the duration of the 'dormant' microscopic stages of the kelp, or how ocean acidification might affect gametogenesis and successful production of sporophytes.

The southern distribution limits of cold-water algal species can be characterized by summer isotherms (Setchell, 1915, 1920), where critical maximum summer temperatures limit growth, reproduction, or survival (van den Hoek, 1975; 1982; Yarish *et al.*, 1984; 1986; Breeman, 1988). Seaweed populations located at the edge of their distribution can adapt to stressful summer conditions (Humm, 1969), but the existence of populations at these boundaries depends on individual temperature tolerances or reproductive requirements (Hutchins, 1947). North Atlantic summer isotherms show considerable interannual variability.
with a general northward trend since 1993, occurring at northern limits with an increasing frequency (Hobson et al., 2008).

Long Island Sound currently experiences summer surface temperatures up to 23-25°C (2012, see fig. 2), with sustained temperatures above 20°C for 6 weeks or more, while the Gulf of Maine has a lower maximum summer temperature, reaching up to 18-22°C. Projected increases of sea surface temperature will push the summer maximum above upper survival temperatures for gametophytes and sporophytes of *Saccharina latissima* in Long Island Sound, and could stress populations in the Gulf of Maine. The upward trend of higher summer temperatures could impact the distribution of *Saccharina latissima* in the Northwest Atlantic, shifting the southern distributional limit north of Long Island Sound.

![Figure 2. NOAA National Data Buoy Center data for 2012. Graph shows actual daily afternoon recorded water temperatures. Gulf of Maine (GOM) buoy 44007, 12 NM Southeast of Portland, ME (http://www.ndbc.noaa.gov/station_page.php?station=44007). Long Island Sound (LIS) buoy 44039, Central Long Island Sound (http://www.ndbc.noaa.gov/station_page.php?station=44039).](image)

The objectives of this study were twofold; to compare two distinct populations from different seasons from the Gulf of Maine and Long Island Sound to determine if there were any ecotypic differences in upper survival temperature thresholds for gametophytes and juvenile
sporophytes, and to investigate effects of increased carbon dioxide and reduced pH on the gametophyte and juvenile sporophyte stages.

The microscopic stages of these two populations from summer and fall were compared to investigate differences in response to projected increases of \( pCO_2 \) and temperature. Impacts on meio-spore settlement, germination, growth, male to female ratios, and reproductive success were investigated under multifactorial temperature (16, 19, 22, 25 & 28°C) and dissolved inorganic carbon (400, 800, 1200, & 1600ppm) conditions.

**Methods and Materials**

Three separate trials were conducted with spores obtained from wild-collected sporophytes from Maine and Long Island Sound, and are represented as; Maine summer (MES), Maine Fall (MEF), and Long Island Sound Fall (LISF). All three trials were successful, but a fourth, the Long Island Summer population trial, was not successful after two separate attempts, due to contamination in each trial by predatory protozoa.

Reproductive plants were collected by snorkeling at low tide at approximately 5-7 meter depths near Simonton Cove in South Portland, Maine, (coordinates 43.6415; -70.2236) in July and September of 2011, and by SCUBA at Black Ledge (coordinates 41.3088; -72.0648) in Long Island Sound in October of 2011 (fig. 3). Reproductive fronds were immediately processed by excising reproductive sorus tissue and scraping, rinsing, scrubbing, and wiping clean, and then placed between layers of clean damp paper towels. Plants were transported to the lab on ice and kept in the dark at 10°C overnight.

Sorus tissue was submerged in 10°C sterilized seawater 20-23 hours after sorus processing for spore release. Three hundred cut glass microscope slides (25x25mm) were inoculated by pipetting 10 mL of spore solution into petri dishes containing 25mL of half-strength Provasoli’s Enrichment Seawater (PES/2) (Provasoli, 1968). The spores were allowed to settle undisturbed and germinate on the slides for 48 hours at 15°C.
Temperature gradient tables at the UCONN Stamford Marine Biotechnology Lab (Yarish et al., 1979) were used to create 20 different environmental conditions of temperature and $p$CO$_2$ values. Dishes were placed on five rows of temperature levels that were set at 16, 19, 22, 25, and 28°C, and four levels of $p$CO$_2$ (at 1x, 2x, 3x and 4x ambient atmospheric levels) were bubbled in the dishes. Each condition of temperature and $p$CO$_2$ had three total replicates, randomly arranged on the gradient table, for a total of 60 dishes.

Each dish was aerated with the appropriate $p$CO$_2$ level of air, which was mixed in 13-liter glass stoppered Pyrex carboy bubbling bottles by combining filtered ambient air and varying levels of CO$_2$. Approximate levels of $p$CO$_2$ were set at 400 ppm, which is an average ambient atmospheric concentration, and 800 ppm, 1200 ppm, and 1600 ppm. These four levels corresponded to average pH values of 7.6, 7.7, 7.8, and 7.9. Air-CO$_2$ mixtures were determined with an Extech CO250 CO$_2$ analyzer, and pH and temperature determined by an Accumet pH meter.

Lighting above the tables consisted of cool white high output T-12 fluorescent bulbs set at a photon fluence rate of 20 µmol photons m$^{-2}$ s$^{-1}$ during the first week, 40 µmol photons m$^{-2}$ s$^{-1}$ during the second week, and 60 µmol photons m$^{-2}$ s$^{-1}$ the third week, based on investigations on light requirements for growth and induction of fertility of gametophytes (Lee & Brinkhuis, 1988, Lüning, 1980, Egan et al., 1989) and optimal development of juvenile sporophytes (Lee & Brinkhuis, 1988, Fortes & Lining, 1980). Photon flux density was measured with a LI-250 light meter (LI-COR, Nebraska). Photoperiod was day neutral at 12:12; L: D.

Percent germination under each condition was determined by pipetting 20 mL of spore solution onto cut glass slides in 150 mL PES/2 sterilized seawater in deep petri dishes at each condition of temperature (16, 19, 22, 25, 28°C) and $p$CO$_2$ (400, 800, 1200 & 1600 ppm). The deep petri dishes were gently aerated at the surface of the water in each dish so that the pH levels could be established without greatly agitating the water. Spores were allowed to settle and germinate in each condition on the gradient table for 48 hours and percent germination was
determined on each slide on the basis of germ tube production per 100 randomly selected spores. All of these slides were removed and new dishes were prepared for the culture of gametophytes that were previously germinated at 15°C. These slides were rinsed with sterilized seawater and placed in deep storage petri dishes (300 mL) on the gradient table at each condition of temperature and pH in PES/2. Germanium dioxide (GeO₂) was added for the inhibition of diatoms (Lewin, 1966).

Seven days after placing germinated spores on the gradient table, one slide was randomly chosen from each dish and photographed under a compound microscope to record growth. This was done again at day 14 (week 2), day 21 (week 3), and day 28 (week 4). Survival, growth, sex ratio, fertility, and sporophyte production and growth were monitored for each condition of temperature and atmospheric CO₂. Temperature and pH measurements were taken at the end of each week with an Accumet pH meter. The meter was calibrated with 6, 8, and 10 pH buffers before measurements were taken. All dishes were changed once per week and slides were transferred into fresh PES/2 + GeO₂ seawater media after each dish had equilibrated to the appropriate temperature and pH level.

Photographic images were analyzed with ImageJ software (Abramoff et al., 2004) for germination, primary cell diameter, female total length, fertility and reproductive success, and sporophyte lengths. 50-100 measurements from each dish were taken for germination, primary cell, length and width measurements.
Figure 3. Map of Northeast Sea Surface Temperatures for 08/02/2013, showing sample collection sites (stars). Adapted from NOAA/NESDIS Geo-Polar Blended 5km SST Analysis for the North Atlantic, at http://www.ospo.noaa.gov/data/sst/contour/natlanti.cf.gif.

**Statistical Analysis**

For each population measure full factorial Analysis of Variance (ANOVA) models were estimated to identify any interactions between factors of pH, temperature, and weeks where applicable. To correct for potential pseudo-replication, the average of all measurements taken within each dish were calculated and used for analysis. Only results from temperature levels of 16°, 19°, and 22°C were analyzed because there were no results at temperature levels 25° and 28°C due to mortality. Model-derived least square (adjusted) means and 95% confidence intervals were subsequently estimated. Variables were log transformed to meet assumptions when necessary. For variables that were log-transformed, least square means were reported as
geometric means after exponentiation. Residual analysis was conducted for all statistical models to verify the assumptions of normality and homogeneity of variance. Analysis of Covariance (ANCOVA) was used to adjust for nuisance variables, when initial results indicated that covariates were obscuring the results. For variables where a suitable transformation could not be found, the Kruskal-Wallis non-parametric ANOVA was used when there were more than two groups being compared and the t-test (assuming unequal variance) was used when comparing two groups. Least square means are plotted as scatter plots, where the individual dots are the estimated means and the error bars are the 95% confidence intervals. All analyses were performed using SAS® 9.3 (Cary, NC) and two sided statistical significance was considered at p<0.05 unless otherwise noted.

Results

Germination

Percentage germination for each replicate was determined by counting the total number of germinated spores, which are meiospores that have attached and developed a germ tube, per 100 randomly selected individuals 48 hours after inoculation of dishes on the gradient table.

Temperature effects on germination were highly significant in all trials (P<0.0001). Data from all three trials were analyzed for normality and variance using residual diagnostics. There were no violations of the assumptions of normality or variance for trials 1 (Maine summer) and 3 (Long Island Sound Fall), but there was substantial heterogeneity of variance for trial 2 (Maine fall). To further investigate this finding, a t-test for unequal variances was conducted, comparing values at 16° and 19° together to 22°C, indicating a significant effect of temperature on germination (P=0.001).

Average germination rates were similar at temperature levels 16°C and 19°C, at around 95%, with no effects of pH. Germination rates dropped drastically at 22°C, with great variability
between replicates, pH levels, and trials, and rates were 0% at 25 and 28°C due to mortality (fig. 4).

Effects of different pH levels on germination were significant only in the Maine summer trial (P = 0.021), but not significant for either the Maine fall (P=0.92) or Long Island Sound fall (P=0.55) trials. Interactive temperature and pH effects were significant for the Maine summer trial (P=0.02), but not for the Maine or Long Island Sound fall trials (P=0.78 and P=0.73).

Results from all three trials show similar temperature effects on germination (fig. 3). The first trial (Maine-summer) had the least variability between pH levels at 22°C, and also the lowest germination success, all which fall below 40%. Trials 2 (Maine-fall) and 3 (Long Island Sound-Fall) had higher germination success at 22°C (between 30-90%), with increased variability among the four different levels of pH. There were no significant effects of pH on germination rates for any of the trials.

![Plot of Percent Germination by Temperature grouped by pH](image)

**Figure 4.** Percent germination by temperature grouped by pH for all three trials.
Female Gametophyte Diameter

Female gametophyte diameter was measured as the diameter of the female primary cell 7 days after germination (fig. 5). Data were log transformed and the geometric mean was used for plotting results, omitting temperature levels 25° and 28°C due to full mortality.

Temperature effects on female cell diameter were highly significant in all trials (P<0.0001). Maine summer and fall diameters were very similar at 16° and 19°C, where values were only slightly larger by approximately 1 micron on average at 16°C for both trials. The Long Island Sound trial produced larger female gametophytes than either of the Maine trials at all temperature levels, and gametophytes were slightly larger at 19°C than at 16°C. Diameters were approximately 4 microns smaller in all trials at 22°C than at 16 or 19°C. Maine fall diameters were slightly larger than the Maine summer trial. There were no significant effects of pH or pH-temperature interactive effects on gametophyte diameter for any of the trials.

Figure 5. Female gametophyte diameter (μm) at one week after germination.
**Female Gametophyte Length**

Female gametophyte length was measured as the total length at the longest point of randomly selected female gametophytes. Data were log transformed due to slight non-constant variance and checked for normality. Results are presented using adjusted geometric means, omitting temperature levels 25°C and 28°C due to full mortality (figures 6-8).

Temperature effects on female gametophyte length were highly significant in all trials (P<0.0001). Total length measurements of female gametophytes were very similar at temperatures 16°C and 19°C for all trials, with values only slightly lower at 19°C than at 16°C, a difference that was more pronounced in the Maine summer trial (fig. 6) than the two fall trials. Geometric mean growth rates over three weeks were highest at 16°C in all three trials, though rates were only slightly lower at 19°C (table 1). Females were significantly shorter at 22°C in all trials, with slower growth rates, the Maine summer trial showing the lowest rates at 14%, and the two fall trials showing similar rates at 70% and 69%.

There were significant effects of pH (P=0.002) on length in the Maine fall trial (figure 7), but not in the other two trials. In some cases gametophytes exposed to the lower pH values (7.6 & 7.7) were slightly longer than the higher pH levels (MES at 19°C, weeks 1-3, MEF at 19°C, weeks 2-3, and LISF at week 2). There were no significant interactive effects of pH and temperature in any of the trials.
Figure 6. Female Gametophyte Length (µm) for Maine summer trial at weeks 1-3.

Figure 7. Female Gametophyte Length (µm) for Maine fall trial, 1-3 weeks.
Figure 8. Female gametophyte length (µm) for Long Island Sound trial, weeks 1-3.

Table 1. Table of rate of geometric mean growth rate of female gametophytes

<table>
<thead>
<tr>
<th>Population</th>
<th>Temperature (°C)</th>
<th>Geometric Mean Growth Rate (% Increase/Week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME Summer</td>
<td>16</td>
<td>110%</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>14%</td>
</tr>
<tr>
<td>ME Fall</td>
<td>16</td>
<td>122%</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>119%</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>70%</td>
</tr>
<tr>
<td>LIS Fall</td>
<td>16</td>
<td>124%</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>108%</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>69%</td>
</tr>
</tbody>
</table>
**Cell Number of Female Gametophytes**

Cell number was determined by counting the number of cells of randomly selected female gametophytes for weeks 1-3. Data were log transformed due to skewed distribution. Temperature effects on female gametophyte cell number were highly significant in all trials (P<0.0001). All gametophytes at 16° and 19°C developed an average increase in cell number from week one to week three, increasing cell number by approximately two cells per week (figs. 9-11). There was very little growth and much less variability at 22°C, with most values at 1 cell and fewer at 2 cells. Median values increased more from week 1-2, with a slower increase from week 2-3. Values ranged from 1 to 31 cells per individual, and unicellular gametophytes could be found at all weeks. Gametophyte size was highly variable, ranging from a unicellular individual to a short filament to a many-celled cluster of cells at both 16° and 19°C and at all pH levels.

There were significant effects of pH (P=0.001) on cell number in the Maine fall trial (fig. 9), but not in the other two trials. Maine and Long Island Fall values were very similar at 22°C, with more cells produced at lower pH values (7.6, 7.7, 7.8) than at 7.9. This trend was not seen in Maine summer values (fig. 8), where most gametophytes were only one cell. Effects of pH were variable, with no obvious trend.
Figure 9. Cell number of female gametophytes, Maine summer trial.

Figure 10. Cell number of female gametophytes, Maine fall trial.
Male: Female Ratio

Male: female sex ratio was determined by counting total number of males and females from 100 randomly selected individuals during week 2 of each trial. Total number of males was divided by total number of females, with a number of 1 representing equal numbers of males and females, n>1 = more males than females, and n<1 = more females than males. The Kruskal-Wallis test (table 2) was used to determine if temperature effects for mean values were significant. Temperature had a significant effect on male to female ratios for the Long Island Sound trial, but not on the other two trials. Gametophytes at 22°C did not develop enough to determine sex, and there was full mortality at temperatures of 25 and 28°C, so only ratios at 16°C and 19°C are reported.

There were no consistent effects of pH on Male: Female sex ratios. Average values for the Maine summer trial clustered around 1.0 at 16°C, and showed a greater range of values at
19°C, from 0.8 to 1.2 (fig. 12). Maine fall values ranged from 0.7-1.1 at 16°C, and 0.8-1.2 at 19°C. The LIS fall trial ranged from 0.6-1.1 at 16°C, indicating a trend towards more females than males, but this result was more equally spread around 1.0 at 19°C. Overall, there was a tendency towards n<1, indicating slightly more females to males within all trials.

Figure 12. Male: Female sex ratio plot (Male/Female gametophytes per 100)

Table 2. Kruskal-Wallis Test for Male: Female ratio results

<table>
<thead>
<tr>
<th>Kruskal-Wallis Test</th>
<th>FLIS</th>
<th>FME</th>
<th>SME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>10.0833</td>
<td>0.0953</td>
<td>0.0038</td>
</tr>
<tr>
<td>DF</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pr &gt; Chi-Square</td>
<td>0.0015</td>
<td>0.7575</td>
<td>0.9509</td>
</tr>
</tbody>
</table>
Fecundity

Fecundity is a measure of reproductive females. Fecundity was determined as the ratio of total fertile female gametophytes, which included eggs and sporophytes, to the total females present at 14 and 21 days after germination. The fecundity ratio is a measurement of the development and degree of gametogenesis, where females become reproductive and begin to release eggs for fertilization.

Temperature was highly significant for all trials. Gametogenesis was not observed at temperatures of 19° or 22°C. There were no significant effects of pH or pH and temperature interactions on fecundity (table 3). Since pH did not have a significant effect on fecundity, all values were plotted across the range of actual observed temperatures. Observed temperatures were plotted to visualize temperature effects, to observe effects of fine scale temperature differences experienced on the gradient table (fig. 13).

The gradient table had a range of values around the set temperature level, and there was an interesting pattern of decreasing fecundity values with increasing temperatures within the range of 15-17°C, with no gametogenesis occurring above 17.2°C (table 4). Fecundity increased with time, from 2 weeks to 3 weeks, for all trials. Maine summer and fall trials exhibited similar values for both weeks, while the Long Island Sound had lower values of fecundity overall for both weeks.

<table>
<thead>
<tr>
<th>Effect</th>
<th>MES</th>
<th>MEF</th>
<th>LISF</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.9221</td>
<td>0.8602</td>
<td>0.8653</td>
</tr>
<tr>
<td>weeks</td>
<td>0.0524</td>
<td>0.0033</td>
<td>0.3141</td>
</tr>
<tr>
<td>pH. / weeks</td>
<td>0.9747</td>
<td>0.9662</td>
<td>0.9921</td>
</tr>
<tr>
<td>Temperature</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0018</td>
</tr>
<tr>
<td>pH. / temp</td>
<td>0.9647</td>
<td>0.902</td>
<td>0.9102</td>
</tr>
<tr>
<td>Weeks / temperature</td>
<td>0.0089</td>
<td>&lt;.0001</td>
<td>0.2749</td>
</tr>
</tbody>
</table>

Table 3. ANOVA results for fecundity
Figure 13. Fecundity values plotted with observed gradient table temperature values

Table 4. Observed critical limits for female gametogenesis

<table>
<thead>
<tr>
<th>Trial</th>
<th>Week 2 (°C)</th>
<th>Week 3 (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME Summer</td>
<td>17.1</td>
<td>17.2</td>
</tr>
<tr>
<td>ME Fall</td>
<td>16.8</td>
<td>16.9</td>
</tr>
<tr>
<td>LIS Fall</td>
<td>16.8</td>
<td>17</td>
</tr>
</tbody>
</table>
Reproductive Success

Reproductive success was determined as the ratio of sporophytes to the total number of female gametophytes 14 and 21 days after germination. Different levels of pH had no consistent effect on reproductive success, though the lowest pH (7.6) resulted in slightly more sporophytes than the other pH levels in the LIS fall trial. Reproductive success increased from week 2 to week 3 at 16°C, and sporophytes were not produced at temperatures above 16°C. All trials showed similar patterns, with fewer sporophytes produced during the LIS fall trial than the two other ME trials (fig. 14).

Figure 14. Reproductive success for all trials at 2 and 3 weeks
**Sporophyte Length**

Sporophyte length was determined from temperature level 16°C (there were no sporophytes produced at 19°C or above) in week three and week four (fig. 15). Values were log transformed to account for exponential growth from week 3 to week 4. ANCOVA was used to determine the effect of the actual temperatures on the gradient table, so observed temperature was initially included as a covariate. Observed temperature did not have significant interaction effects with weeks or pH, indicating that the observed gradient temperature was not a significant factor and was dropped from the model. Re-running the analysis without the observed temperature showed weeks as the only significant factor (P < 0.0001), while pH and the pH x Weeks interaction were insignificant (P= 0.7886 and P=0.3372 respectively). Geometric growth rates were calculated to look at relative growth from week 3 to 4 at the four levels of pH at 16°C (table 4). The lowest pH (7.6) had the highest growth rate, while pH 7.8 had the lowest growth rate.

![Figure 15. Geometric mean of juvenile sporophyte length for all values at weeks 3 and 4.](image-url)
Table 4. Geometric mean growth rates for juvenile sporophytes at 16°C

<table>
<thead>
<tr>
<th>pH</th>
<th>Geometric Mean Growth Rate (% Increase/Week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6</td>
<td>345%</td>
</tr>
<tr>
<td>7.7</td>
<td>147%</td>
</tr>
<tr>
<td>7.8</td>
<td>63%</td>
</tr>
<tr>
<td>7.9</td>
<td>171%</td>
</tr>
</tbody>
</table>

Discussion

Temperature is a major environmental factor controlling the success of microscopic stages of *Saccharina latissima*, where major changes in survival, growth, and gametogenesis can occur within just a few degrees of the upper limits, while decreases in seawater pH have slight and variable to no impacts. Gametophytes were cultured at five temperature levels (16°, 19°, 22°, 25°, 28°C), representing a range of optimal to lethal temperatures for the microstages of *S. latissima*. Gametophytes experienced full mortality in all three trials at temperatures of 25° and 28°C, with 22°C representing an upper critical limit, with significant mortality and little growth or development of gametophytes. The same patterns of temperature response (slightly higher or similar values for 16°C compared to 19°C, with a large decrease in values at 22°C) were observed in all three trials for percent germination, primary cell diameter, and female length and cell numbers over three weeks.

Germination and male and female growth were very similar at 16° and 19°C, but greatly reduced at 22°, and full mortality at the higher temperatures of 25° and 28°C. This indicates that there is a critical shift in the ability of spores to germinate and develop at 22°C, an upper limit where cells are under significant temperature stress. This value is slightly lower than reported values within the range of 23-25°C (Egan et al., 1989; tom Diek, 1993), but is within the 18-23°C range reported by other studies (Lüning, 1980; Bolton & Lüning, 1982; Kain, 1969; Breeman, 1988; Lüning, 1984). Growth rates of female gametophytes at 16° and 19° were similar, but
gametogenesis was completely inhibited above 17°C for all trials. This is similar to values reported by other studies (Egan et al., 1989; Bolton & Lüning, 1982; Yarish & Egan, 1989; Lüning, 1984; Lee & Brinkhus, 1988, see table 5). All gametophytes increased in size over time.

Table 5. Comparison of reported critical temperature limits (°C) for *Saccharina latissima*.

<table>
<thead>
<tr>
<th>Study</th>
<th>Germination</th>
<th>Growth</th>
<th>Gametogenesis</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>optimal</td>
<td>max</td>
<td>range</td>
<td>upper</td>
</tr>
<tr>
<td>Egan et al., 1989</td>
<td>5-15</td>
<td>25</td>
<td>10-20</td>
<td>25</td>
</tr>
<tr>
<td>Lee and Brinkhuis, 1988</td>
<td>10 - 20</td>
<td></td>
<td></td>
<td>7-14</td>
</tr>
<tr>
<td>Lüning, 1980</td>
<td>10 - 19</td>
<td>20 - 21</td>
<td>22</td>
<td>10-15</td>
</tr>
<tr>
<td>Yarish &amp; Egan, 1989</td>
<td>10 -20</td>
<td></td>
<td></td>
<td>5-20</td>
</tr>
<tr>
<td>Tom Diek, 1993</td>
<td></td>
<td>23-25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kain, 1969</td>
<td></td>
<td></td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Schreiber, 1930</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Druehl, 1967</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harries, 1932</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeman, 1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lüning, 1984</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>22</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Population response differed, most notably between the female primary cell diameters. Long Island Sound cultures were larger than Maine trials at all temperatures, and gametophytes at 19°C were slightly larger than at 16°C, where the reverse was true for both Maine trials. Gametophytes obtained in the summer months had lower germination success, smaller primary cell diameters, shorter lengths, and fewer cells at 22°C than both fall trials, indicating a seasonal difference. Altering pH did not result in any obvious or consistent effects though lower pH values
(7.6-7.7) did result in slightly better growth (primary cell diameter; MES and MEF at 16 and 19°C, gametophyte length: MES at 19°C, MEF and LISF at 16 and 19°C) than the highest value (7.9).

Ecotypes are populations within a species that have adapted to local conditions, displaying differential responses to given environmental variables. Several studies have attempted to identify *Saccharina latissima* ecotypes, with results ranging from no evidence of ecotypic response (Breeman, 1988; Lüning, 1984) to distinct differences in upper survival temperatures between geographically distinct populations (Gerard, 1990; Bruhn & Gerard, 1996; Lüning, 1975, Lüning *et al.*, 1978; Müller *et al.*, 2008). Seaweed populations from the southernmost portion of their distribution often exhibit higher temperature tolerance (Breeman, 1988), and this has been observed in gametophytes and sporophytes from Long Island Sound (Egan *et al.*, 1990; Gerard, 1990), though in my study the upper critical temperature limit was the same for the Maine and Long Island Sound populations, at 22°C. There were small differences between the two populations, however, in primary cell diameter, where Long Island Sound female gametophytes were larger in diameter than the Maine gametophytes at all temperatures.

Water temperature is the most important factor determining distribution of seaweed species (Lüning, 1990), so a predicted increase of seawater temperature could shift the lethal, reproductive, and growth limit isotherms for *Saccharina latissima* northward (Bartsch, *et al.*, 2008), though no major latitudinal shifts have been observed to date in the subarctic and boreal zones in the Western Atlantic in the past century (Merzouk & Johnson, 2011). Southern Maine kelp populations experience upper summer maximum temperatures of 21-23°C, with sustained temperatures of 18-20°C (fig. 2). An increase of 2°C above 19°C could be physiologically stressful or lethal for southern Maine and New England populations of kelp sporophytes.

Vegetative growth of gametophytes will likely increase at temperatures above 15°C, with an inhibition of gametogenesis above 17°C (this study; Lüning, 1980; Lee & Brinkhuis, 1988; Bolton
& Lüning, 1982; Harries, 1932; Druehl, 1967; Yarish et al., 1990), and increase in mortality above 22°C (this study; Lüning, 1980; Bolton & Lüning, 1980). Growth rates of sporophytes could increase for kelp populations located in the middle and upper latitudes up to a point (Coelho et al., 2000), since photosynthetic rates rise with increasing temperatures, but above optimal temperatures, rates decline rapidly (Druehl, 1967).

Distributional boundaries are coinciding extremes of environmental conditions. A sustained, viable kelp population depends on water temperatures that do not either exceed individual critical survival temperatures of sporophytes or gametophytes, or inhibit the settlement, germination, or reproduction of gametophytes. In Long Island Sound, the warm temperature Western Atlantic boundary for Saccharina latissima, sea surface temperatures have already increased an average of 1°C in just over 2 decades (1979-2002), with summer temperatures peaking at 22-23°C (Keser et al., 2005). Summer seawater temperatures in Western Long Island Sound are already 2-3 degrees higher than temperatures in Eastern Long Island Sound, (Howard-Strobel et al., 2000), so it would be interesting to compare the presence or absence of kelp beds in Western Long Island Sound compared to Eastern Long Island Sound to evaluate the impact of summer temperatures rising above the 22°C upper critical thermal limit. As this warming trend is predicted to continue (IPCC, 2013), with an increase of 3°C by the end of the century, maximum summer temperatures in all of Long Island Sound are likely to exceed upper survival temperatures for sporophytes and gametophytes.

Sporophytes are annuals or biennials in Long Island Sound (Pedersen et al., 2008), which means that either sporophytes or gametophytes must survive the summer temperatures to produce new generations every year (Müller et al., 2009). Upper survival temperatures are 1-5°C higher for gametophytes than sporophytes, so it is likely that the gametophyte stage is the dominant survival stage. Lee & Brinkhuis (1986) first suggested that gametophytes were the over summering stage in Long Island Sound, but later retracted that hypothesis in favor of sporophytes (1988). They observed that first year sporophytes were most evident in the field in
May-June and early autumn, but whether these are from previously existing microscopic stages or new recruits is unknown. The peak reproductive periods for sporophytes occurs in the fall (September-October) at periods of decreasing temperatures of 18-8°C (Van Patten & Yarish, 1993), which would produce young sporophytes for the late fall and winter season, but would not account for the early fall sporophytes.

High summer temperatures may significantly affect meiospore germination, though there is evidence of seasonal acclimation to germination (Egan et al., 1989). Meiospore germination success was similar across both Maine and Long Island Sound populations and both summer and fall trials at temperatures of 16-19°C, but was greatly reduced at 22°C. Both fall trials were more successful (~60%) than the summer trial (~20%).

Southern range gametophytes would likely be vegetative throughout the summer at temperatures of 17-22°C, which typically occurs between June-July and September-November in Long Island Sound. Gametogenesis was initiated only at temperatures below 17°C in this study, and high levels of mortality experienced above 22°C. Egan et al. (1989) suggested that exposure to high temperatures in summer could suppress development and growth of microscopic stages. However, there are other factors that can influence gametogenesis, including interacting factors of light, temperature, temperature history, and nutrient availability. Initiation of gametogenesis at higher temperatures (>15°C) requires exposure to higher photon flux than at lower temperatures (Lüning, 1980), so it is possible that gametogenesis occurs at summer temperatures above 17°C. There could also be a seasonal temperature acclimation for fecundity (Costa, 1989), as observed in trials of acclimated vegetative gametophytes cultured in the lab (Bolton & Lüning, 1982).

For mid range populations, gametogenesis has been observed to occur at all times of the year in areas of maximum summer temperatures of 16°C (Hsaio, 1972). In these populations, the establishment of new macroscopic sporophytes is limited not by gametogenesis but by response of the juvenile sporophytes to environmental factors (Hsaio,
Maximum summer temperatures in the Gulf of Maine are consistently above 16°C from July to September, fluctuating from 16-20°C. Both gametogenesis and vegetative growth probably occurs throughout the summer in the Gulf of Maine, with the production and success of macroscopic sporophytes a function of the interaction of physical environmental variables and other biological variables, such as competition and herbivory.

While male and female gametophytes are released at a 1:1 ratio (Schreiber, 1930), there have been variable results reported for M:F sex ratios at higher temperatures. More males than females have been observed at higher temperatures (Lee & Brinkhus, 1988; Kain, 1979; Bolton & Lüning, 1982), but females had a slightly higher upper survival temperature (tom Dieck, 1993), and have been observed to be more prevalent under stressful conditions (Uller et al., 2007). The M:F ratio ranged around the typical 1:1 ratio at temperatures of 16-19°C, temperatures within the “broad range” of S. latissima, though gametophytes at 22°C did not develop enough to determine sex.

Egan et al. (1989) suggested that the summer strategy for gametophytes is to produce sporophytes rapidly, after observing new recruitments in LIS just one month after setting lines out in eastern coastal Long Island Sound in June of 1985. Yarish (unpublished data) has also observed new recruits in western and central Long Island Sound on lines set out in 2012 and 2013. Kelp meiospores have a limited range of dispersal (Dayton, 1985) so new recruits indicate the presence of a reproductive population somewhere within the vicinity. Summer survival and presence of new recruits throughout the year may be an indication of possible deep-water kelp refugia populations within Long Island Sound. Deep water kelp forests have recently been discovered in tropical waters where warm temperatures and low nutrient levels are not otherwise adequate for kelp survival (Graham et al., 2007). Mathieson (1979) observed Saccharina latissima populations at 26 meters depth in New Hampshire, and found that deeper water sites supported more perennial populations compared to shallower sites with a wider range of temperatures. Transplantation experiments also observed positive net growth
throughout the summer at depths of 6, 9 and 12 m, compared to a net decrease in shallow water individuals (Boden, 1979). Deep water populations may be able to support sporophyte populations through stressful summer temperatures with colder, more stable temperatures and higher levels of nutrients available below the thermocline (van den Hoek, 1982).

Mean surface sea temperatures are a very general measurement of actual water temperatures, averaged over time. The upper surface layers of the ocean are typically warmer in the summer than the deeper, colder layers experienced by subtidal populations (van den Hoek, 1982). While experimental temperatures can provide some insight into critical upper limits, the ability for southern limit populations to persist above upper limits may be due to lower actual temperatures experienced by deeper populations, or their ability to withstand short-term exposure to higher temperatures. Macroalgae can often recover from short term exposure to stressful super-optimal temperatures, but as overall temperatures rise, there will be increasing episodes of exposure of longer durations to upper survival temperatures, and populations will need to adapt to these exposures, or will disappear from these areas.

The increase in dissolved seawater carbon concentrations predicted for this century and the resultant decreases in pH are not likely to directly impact *Saccharina latissima* gametophytes. There were no obvious effects of decreased seawater pH on the microscopic stages in this study, even up to 4 times higher than current levels. Subtidal kelp populations already experience local variability and exposure to low levels of pH in coastal areas, due to physical (e.g. upwelling, freshwater inflows, temperature, tidal exchange) (Hunt et al., 2011), seasonal (e.g. lower pH values during winter) (Hsaio, 1972), and daily natural cycles from the biological processes of photosynthesis and respiration (e.g. 2.4 – 1.05 units over one day (Wootton et al., 2008, Delille et al., 2000)). Indirect effects of decreased pH could include changes in seawater chemistry and nutrient availability (e.g. increased iron bioavailability (Breitbarth et al., 2010)) or decreases in herbivory pressure by impacting key shell-forming predators (i.e. echinoderms and snails).
Areas of natural local acidification from underwater volcanic vents show overall shifts in benthic community composition, with a decrease in overall biodiversity. Algal groups in these pH-shifted environments are successful, while coralline species (corals and sea urchins) decline and disappear along gradients toward the vents as CO$_2$ levels increase (804-957µatm pCO$_2$) and pH levels decrease (7.8-7.4) (Hall-Spencer et al., 2008). Green sea urchins (Strongylocentrotus droebachiensis) and the herbivorous snail Lacuna vincta are major algal herbivores of kelp and could be negatively affected by a changing ocean climate. High densities of sea urchins can reduce productive kelp beds to bare urchin and encrusting coralline algal communities, while a decrease in urchin population will allow a re-colonization of the kelp canopy. Kelp forests and urchin barrens have historically been the two major Northwest Atlantic subtidal community types, the dominant community type controlled by the increase or decrease in urchin population (Chapman & Johnson, 1990). Ocean acidification and increased temperatures have negative effects on tropical larval sea urchins, resulting in decreased biomineralization of the larval skeleton (Brennand et al., 2010). Epizootic events causing widespread urchin mortality is associated with warmer summer temperatures and more frequent tropical storm and hurricane activity in Nova Scotia (Scheibling et al., 1999). A decrease in urchin or snail abundance could reduce grazing pressure on kelp and result in more stable kelp dominated communities with fewer herbivory-related disturbances.

The combined effects of increased temperature, increased CO$_2$, and increased nutrient availability in coastal waters (Connell & Russell, 2010; Russell et al., 2009) are predicted to contribute to changing algal communities, with an increase in fleshy, ephemeral, foliose and filamentous macroalgae, and a decrease in calcifying algae. This shift can be seen in areas of natural acidification near underwater vents (Hall-Spencer et al., 2008), where erect and crustose calcified species are replaced by non-calcified algae in areas of lowered pH, resulting in an overall simplification of the macroalgal community (Porzio et al., 2011). Red algal species are predicted to increase in abundance with an increase in temperature (Schiel et al., 2004), and
these effects have been observed in Long Island Sound with declines in intertidal Phaeophyceae and simultaneous increases in Rhodophytes (Pedersen et al., 2008). There have already been increasing occurrences of invasive algal species in New England [e.g. Heterosiphonia japonica, (Newton et al., 2013), Grateloupia turuturu, (Mathieson et al., 2008) and Codium fragile (Levin et al., 2002)] with broad thermal and salinity tolerances.

Environmental abiotic factors (temperature, currents, nutrient levels, eutrophication, UV exposure, acidification) and biotic factors (competition, grazing, pathogens) can all affect kelp distribution and abundance. These manifold factors can work to have antagonistic or synergic effects on an organism. It is difficult to predict the effects of just one variable on an organism since one factor will likely occur with others. Survival will depend on mechanisms of acclimation, including photoinhibition and photoprotection, nutrient uptake strategies, patterns of growth and reproduction, and morphogenetic responses (Figueroa & Korbee, 2010).

The stress associated with an increase in temperature can act synergistically with other factors, including pests and pathogens. Kelp pathogens can include bacteria, viruses, fungi, oomycetes, bryozoans, and endophytic red and brown filamentous algae. Infections in kelp can weaken plants, reduce fertility, and cause mortality. Increased temperatures could increase susceptibility to disease and cause increased pathogenic infection due to increased algal stress, faster pathogenic development, increased winter survival, and increased host susceptibility (Eggert et al., 2010). Membranipora membranacea is an introduced crust-forming bryozoan that is significantly affecting kelp populations in the Northwest Atlantic (Lambert et al., 1992) and its spread and success is correlated with higher sea temperatures. The encrusting parasite can cover large portions of the blade, resulting in an inhibition of photosynthesis, weakened tissue, reduced spore output, an increase in blade loss and ultimate kelp deforestation (Saier & Chapman, 2004; Andersen et al., 2011; Yarish & Egan, 1987).

The coastal zone is a dynamic system that represents an important link between land, open ocean, and atmosphere. Anthropogenic activities have altered coastal marine systems
directly and indirectly through fossil fuel and biomass emissions, land use change, inorganic fertilizers, and increased nutrient loading. The carbon cycle is linked to other important biogeochemical cycles, including nitrogen, phosphorus, and sulfur, and is affected by riverine and atmospheric inputs as well as biological and physical feedbacks (Mackenzie et al., 2002). The seawater carbonate system will react to changes in any of the individual components, so increases in total alkalinity could buffer the effects of increased CO$_2$ (Egleston, et al., 2010). Alkalinity is a function of riverine inputs, calcification, sedimentation, nitrogen uptake, and other biological and chemical sources and sinks, and riverine inputs to the coastal zone are increasingly alkaline in the Northeast due to acid deposition and terrestrial weathering (Kaushal et al., 2013). Increased nitrogen and phosphorus loading to coastal systems could enhance production, resulting in a downward carbon pump, but organic loading can also cause eutrophication, which results in anaerobic conditions and a decrease in overall productivity. Anthropogenic alteration of global biogeochemical cycles will have cascading chemical, physical, and biological feedback effects that could impact the health of the marine system.

**Conclusions**

Rapid changes in the ocean environment have unforeseen consequences on marine organisms and ecosystems. Temperature and pH are both critical biological variables that can affect survival, growth, reproduction, recruitment, productivity, and biodiversity of a system. These factors all interact in a natural system, and significant changes in these important variables will most likely affect the larger marine ecosystem, which could have both direct and indirect affects on kelp populations worldwide. It can be predicted, however, that increasing temperatures at the southern boundaries will inevitably shift the distribution northward.
References


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