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Effects of Temperature and Nutrient Regimes on the Recruitment of *Laminaria digitata*

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Abstract

Kelps are important macroalgae found in the temperate rocky marine habitats along the northern Atlantic Ocean coast. Kelp forests and its services to the ecosystem are globally at risk due to anthropogenic activity and climate change, by altering the temperature gradient and the nutrient input and where the kelps exist. This could be detrimental for the coming generations of the kelp, *Laminaria digitata,* which is one of the major kelp species in the north Atlantic Ocean. As of this, nutrients and temperature regimes that allow microscopic phases for gametogenesis and survival could be at risk. As gametophyte growth and sporophyte development have different optimal temperatures, the microscopic life phases develop at different rates when exposed to different environmental conditions. To investigate the effects of temperature and nutrient regimes on gametophyte growth, gametogenesis, and early sporophyte formation of *L. digitata*, two populations (Bodø and Quiberon) were exposed to four temperature regimes (4, 8, 12, 18^{o}C) and two nutrient treatments (high (HN) and low(LN)) based on local data from each site. Bodø represents the intermediate zone, which might provide optimum conditions while Quiberon represent the southern distributional limit. The experiment followed a factorial design, testing all temperatures in both nutrient conditions through a mechanistic approach considering both sites. We hypothesized that the response of each population shows local adaptation relating to local nutrient and temperature histories. Results showed that the gametophytes of each population responded differently to the treatments, especially in respect to sporophyte formation, however had similar patterns for development in general. Sporophyte recruitment was 7% higher for the Quiberon population, however, both populations responded positively to optimal temperatures (8 and 12^{o}C) in terms of ontogenetic development. Moreover, both populations interaction with temperature were significantly related to sporophyte recruitment, with Bodø also having a response to the nutrient treatment. In both populations, longer periods of increased temperatures (18^{o}C) enhanced vegetative gametophyte growth but inhibited sporophyte production. This study provides an insight to how *Laminaria digitata* gametophytes responds to forced environmental conditions in terms of survival, growth, and recruitment. It shows distinct differences between populations, exhibiting local adaption based on local nutrient and temperature histories. With emphasis on which treatments results in the optimal conditions to further knowledge on the adaptation responses of the species to environmental conditions and climate change.

Keywords: Kelp gametophytes, local adaptation, temperature, nutrients, recruitment

Resumo

Macroalgas como a *Laminaria digitata*, são algas importantes capazes de fornecer abrigo e recursos a outros organismos na extensão da costa norte do Oceano Atlântico, especificamente em habitats marinhos rochosos temperados. A atividade antropogénica e as alterações climáticas, colocam as florestas de algas e os serviços que estas oferecem ao ecossistema em risco a nível global ao alterar o gradiente de temperatura e a entrada de nutrientes e onde estas existem. Isto poderá ser prejudicial para as próximas gerações de uma espécie de algas, *Laminaria digitata*, a maior das algas no Oceano Atlântico Norte. Este risco está associado ao facto de os nutrientes e os regimes de temperatura que permitem fases microscópicas de gametogénese e sobrevivência poderem estar em risco. Em condições desfavoráveis, os gametófitos têm o potencial de permanecer em crescimento vegetativo. Por sua vez há um fornecimento de um banco de sementes ao fundo do oceano, à espera de condições mais favoráveis. Além disso, em comparação com as plantas terrestres, os gametófitos de algas carecem de cápsulas de sementes para proteção contra o stress local, sendo assim mais suscetíveis a fatores ambientais e perturbações causadas por correntes, herbívoros, e alterações diretas ao seu ambiente local. Algas, tais como *L. digitata*, possuem crescimento de gametófitos e a formação de esporófitos através da gametogénese em intervalos de temperatura diferentes. Isto torna as espécies vulneráveis a períodos prolongados de temperaturas abaixo do ideal, o que pode ter um impacto nos períodos de tempo normais de desenvolvimento e crescimento de gametófitos, e na formação de esporófitos.

Colocamos a hipótese de que a resposta de cada população mostra uma adaptação local relacionada com o registo de ocorrência de nutrientes e temperaturas. Para investigar os efeitos dos regimes de temperatura e nutrientes no recrutamento de *L. digitata*, duas populações (Bodø e Quiberon) ao longo do gradiente de distribuição ótimo e mais meridional foram examinadas sob um conjunto de tratamentos. Os gametófitos foram expostos a quatro regimes de temperatura (4, 8, 12, 18ºC) e dois tratamentos de nutrientes (alto (HN) e baixo (LN)) com base na média local dos dados do verão e inverno de cada local. A experiência seguiu um desenho fatorial, testando todas as temperaturas em ambas as condições nutricionais através de uma abordagem mecanicista considerando ambos os locais. Cada um dos meios nutritivos (HN e LN) foi criado individualmente para ambas as populações, antes do início da experiência em t0. Duas semanas antes do início do processo experimental em água do mar artificial, o material experimental de gametófito vegetativo não foi alimentado. Este procedimento foi aplicado para que os isolados de gametófito se esgotassem em nutrientes, a fim de examinar as concentrações

de nutrientes no meio e o seu efeito no desenvolvimento e crescimento dos mesmos. Posteriormente, para permitir que os gametófitos se aclimatassem à sua temperatura final atribuída no dia 0, a aclimatação foi estabelecida 5 dias antes do início do processo experimental para permitir que os gametófitos recuperassem do stress da sementeira, se ajustassem e replicassem a criação.

Os resultados mostraram que os gametófitos de cada população responderam de forma diferente aos tratamentos, especialmente no que diz respeito à formação de esporófitos, tendo, no entanto, padrões semelhantes para o desenvolvimento em geral. Em Quiberon, havia aproximadamente 20% mais machos do que fêmeas no quinto dia, enquanto que em Bodø, havia aproximadamente 30% mais fêmeas do que machos, tornando-o um artefacto experimental. É provável que a diferença entre a proporção de sexos entre as populações tenha influenciado o recrutamento esporófito final no 20º dia. No entanto, a proporção entre sexos não foi influenciada pela temperatura e condições nutricionais e não houve interação de fatores. Todos os gametófitos apresentavam apenas uma baixa fertilidade independentemente da temperatura e do nutriente, não ultrapassando os 20% para os gametófitos com ovos ou esporófitos. O recrutamento de esporófitos foi 7% mais elevado para a população de Quiberon, contudo, ambas as populações responderam positivamente às temperaturas ótimas (8 e 12ºC) em termos de desenvolvimento ontogenético. A maioria dos esporófitos desenvolveu-se a 8 e 12°C em ambas as populações, enquanto que a 4 e 18°C, os gametófitos cresceram vegetativamente na sua maioria. Além disso, a interação de ambas as populações com a temperatura estava significativamente relacionada com o recrutamento de esporófitos, tendo Bodø também uma resposta ao tratamento com nutrientes. O maior recrutamento de esporófitos por população no 21º dia foi observado a 12ºC sob tratamento HN para Quiberon com 37 esporófitos cm^2. Enquanto que, o máximo para Bodø verificou-se a 8ºC sob tratamento de HN com 11 esporófitos cm^2.

O crescimento do gametófito foi avaliado apenas para a população de Bodø. O crescimento de gametófitos foi significativamente afetado pelo tratamento com nutrientes no final (t20) da experiência: os gametófitos em tratamentos com nutrientes altos cresceram mais do que aqueles sujeitos a tratamentos com nutrientes baixos. Os gametófitos a 18ºC HN não se tornaram férteis, mas cresceram mais do que todos os outros gametófitos sujeitos a outros tratamentos. Além disso, uma vez que alguns gametófitos cresceram em grande escala, levou a que outros estivessem em decomposição, provavelmente causando a libertação adicional de fosfato no meio de cultura e a concentrações finais do mesmo mais elevadas comparativamente aquelas do tratamento inicial. Derivado dos tratamentos com baixos nutrientes sob

temperaturas abaixo dos níveis ótimos, a densidade de gametófitos diminuiu com o tempo, devido à sua deterioração. Os efeitos resultantes da interação população x nutriente e população x temperatura, foram significativos para a densidade de gametófitos no 20º dia. Adicionalmente, o tratamento de temperatura também teve um efeito significativo independente sobre a densidade para ambas as populações. Em condições de HN, a concentração inicial de nitrato não foi totalmente utilizada no prazo de 21 dias, sendo apenas reduzida em cerca de 50% (de 15 para 7-12 μmol L^{-1}), em contraste com o fosfato, que foi totalmente utilizado.

Períodos mais longos de aumento de temperatura (18ºC) têm o potencial de aumentar o crescimento vegetativo de gametófitos, mas atrasam ou inibem a produção de esporófitos. As temperaturas dentro da gama ótima (8-12ºC) para o recrutamento são cruciais para que a espécie persista numa área ao longo do tempo. Com o aumento das temperaturas de verão e as limitações da disponibilidade de nutrientes resultantes das flutuações locais causadas pelas alterações climáticas, as concentrações naturais de nutrientes e as temperaturas anuais podem ser alteradas. Isto poderá levar a que mais gametófitos permaneçam em fase vegetativa, à espera de condições ambientais favoráveis. Assim, como as populações em foco responderam de forma semelhante aos tratamentos de temperatura, as mudanças nas interações das espécies com o ambiente podem ser mais proeminentes, e a sua gama de distribuição pode mudar para fazer face às mudanças no seu ambiente. No entanto, é necessário realizar mais estudos para aprofundar mais conhecimento e consciência sobre a forma como diferentes fatores ambientais afetam a vida biológica e os processos de subpopulação de algas, com mudanças contínuas que estão a ocorrer não só a nível global, mas também a nível local. Este estudo fornece uma perspetiva de como a alga *laminaria digitata* responde às condições ambientais forçadas em termos de sobrevivência, crescimento de gametófitos, desenvolvimento, recrutamento e utilização de nutrientes. Com destaque às variantes de temperaturas e concentrações de nutrientes que resultam em condições ótimas e abaixo de ótimas, visando aprofundar o conhecimento sobre as respostas de adaptação das espécies às condições ambientais e às alterações climáticas

Introduction

Kelps are macroalgae belonging to the order Laminariales, which are brown algae that exists in rocky coastal communities from the northern polar ecosystems to temperate/warm ecosystems (Steneck *et al.* 2002). The order includes a variety of important macroalgae which provide many important ecological, social, and economic importance's to the surrounding flora and fauna with nurseries, shelter, and food (Bennett *et al.* 2016; Martins *et al.* 2017, 2019). Kelps represent high ecological value through its support of the surrounding areas by keeping the sediments still and minimizing the chances of erosion. Additionally, they have the ability to remove pollutants through bioremediation along the coastlines, serving as a protector of ecologically important ecosystems (Bartsch *et al.* 2008; Martins *et al.* 2017). Additionally, Laminariales are becoming increasingly more common in animal and human consumption (Bartsch *et al.* 2008) but are threatened by global warming and other anthropogenetic factors (Martins *et al.* 2017).

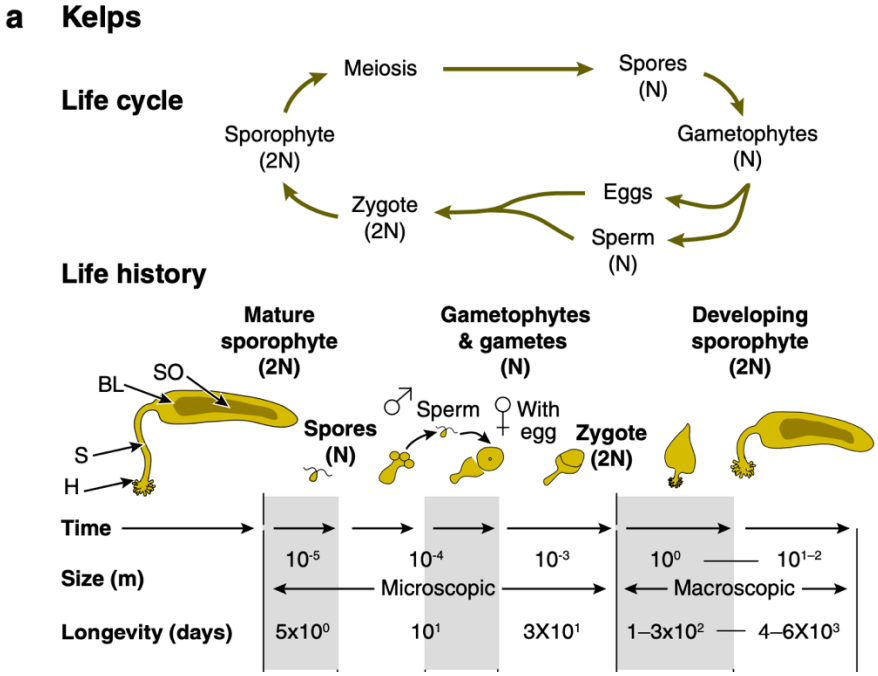

Figure S1: Showing the life phases of Laminariales (Schiel & Foster, 2006)

Laminariales have a haplo-diplontic heteromorphic life cycle and thereby alternates between a microscopic haploid gametophyte phase (1N) and a diploid (2N) phase as microscopic sporophytes (Kain, 1979; Schiel and Foster, 2006). As the sporophytes become fertile (Figure S1), parts of the blade change into sori which produce haploid meiospores. From these meiospores, further development leads to the production of separate male and female (1N)

dioicous gametophytes through germination (Martins *et al.* 2017).
During their ontogenetic development dioicous gametophytes become fertile under favorable conditions of irradiance, temperature, and nutrients (Martins *et al.* 2017). Fertile female gametophytes produce an oogonium which release single eggs which are fertilized by male spermatia. The resulting zygote (2N) is a primordial cell of juvenile sporophytes, which leads to the development of a new generation where the life phases continue. Sexual reproduction under the Laminariales is oogamous, meaning that reproduction is explained by the union of a mobile male and immobile female, by the females' eggs (immobile) releasing the pheromone lamoxirene and attracting spermatozoids (mobile) originating from the antheridium (Maier and Müller, 1986; Müller *et al.* 1979; Schiel and Foster, 2006).

Previously, kelp and other seaweeds were considered to be equivalent to terrestrial plants (Darwin, 1860) due to misunderstandings of algae life histories and more in detail their life cycles (Schiel and Foster, 2006). In contrast to terrestrial seed plants with seed coverings, kelps expose both their haploid (1N) and diploid (2N) stages directly to the environment, thus reflecting the phylogenetic constraints aswell as distinctive characteristics of the environment in which it originates from and is exposed to (Schiel and Foster, 2006). Moreover, due to the microscopic phases being directly exposed they are consequently susceptible to environmental factors of any kind, physical damage, water motion, sedimentation, and consumption by grazers (Morelissen *et al.* 2013; Santelices, 1990). Hence, the success of the development of the microscopic stages are related to the population dynamic of the particular species in focus, which additionally depends on the temperature regimes and nutrient availability. Another note is that kelp beds are recognized as being highly dynamic, existing in environments that vary in terms of essential resources such as sufficient space, nutrients, and light as well as favorable temperature regimes (Barradas *et al.* 2011). However, with the presence of microscopic growth remaining vegetative at the ocean floor, kelp have the capability of enduring acute disturbances such as removal of masses of kelps, to the extent where it can fully recover over a period of months (Barradas *et al.* 2011).

Both sporophytes and gametophytes have different optimal temperatures for growth and ontogenetic development, for instance *Laminaria digitata* has the optimal temperature for growth of gametophytes between 10-18^{o}C (Lüning, 1980) and for sporophytes 5-15^{o}C (Martins *et al*. 2017; tom Dieck, 1992). As gametophyte cells are omnipotent, they have the potential to be transferred into reproductive cells (Martins *et al*. 2017) upon favorable

conditions and delay ontogenetic development. Delayed gametophytes may resume reproduction when under favorable conditions; due to their omnipotency vegetative growth, further growth may enhance the amount of potential reproduction cells (Bartsch *et al.* 2013). Even though the delay during the microscopic stages may be due to unfavorable environmental conditions, delaying the development of the macroscopic stages may be favorable as the microscopic stages are more resistant to unfavorable temperatures than the macroscopic stages (Martins *et al.* 2017; Barradas *et al.* 2011). Thus, by delaying the development into the adult stages, the prospect of successful sporophyte recruitment may be enhanced (Barradas *et al.* 2011).

In addition to what was elaborated above, nutrient conditions are one more major environmental factor influencing the gametophyte development (Forbord *et al*. 2021; Suzuki *et al*. 1994). Moreover, iron deficiency suppress fertility in gametophytes as its interaction with nitrate and phosphate induce the formation of oogonium and female gametophytes (Suzuki *et al*. 1994). In Norwegian coastal waters the nitrate concentration in the main growth season of kelp sporophytes (winter – early spring) varies between 2-18 µM L-1, which is considered to be between low and high concentrations along the Norwegian coastline (Forbord *et al*. 2021). To achieve optimal growth and nutrient utilization for the kelp, knowledge on how extracellular nutrient concentration, uptake processes, and intracellular nitrate concentration affect one another are important in understanding how the environment provides sufficient nutrients for growth to meet the kelp sporophytes nutritional requirements (Forbord *et al*. 2021). In situ nutrient and temperature condition are interlinked and vary together throughout the seasons. Often low nutrient concentrations and high temperatures are characteristic for summer situations (Forbord *et al.* 2021). Thus, it is assumed that sporophyte development and growth among kelps are restricted to more favorable seasons such as winter or spring where temperatures are closer to the optimal range and nutrients are available (Handå *et al.* 2013; Martins *et al*. 2017). However, induction of optimal growth and development for kelps gametophytes tend to require both sufficient temperature and nutrient concentrations to avoid staying vegetative and resume its ontogenetic development (Martins *et al.* 2017). With an increase in coastal nutrients due to anthropogenic activities such as proximity of kelps to aquaculture, sporophyte growth becomes positively influenced (Handå *et al.* 2013), which can aid the growth rate and length of individuals. Some areas along the northern (north of 62^{o}N) Norwegian coast have experienced an increase in nutrient availability (phosphorus and nitrogen) due to aquaculture in addition to the already present natural concentrations of the

Norwegian coastal current (NCC) (Aure and Skjoldal, 2004), yet low enough concentrations as a whole from natural and unnatural sources to avoid eutrophication. Quiberon which lay at the southern distributional limit of the for the kelp, *L. digitata*, have been experiencing a downward trend in nutrient concentrations (kilotons/year) for nitrates and phosphorus since 1997 (OSPAR, 2018). However, annual concentrations of phosphorus (P) and nitrogen (N) are far higher in Quiberon (N: 137 kilotons and P: 12 kilotons) than for Bodø (N: 53 kilotons and P: 4.1 kilotons) (Aure and Skjoldal, 2004; OSPAR, 2018) .

The genus *Laminaria*, within the order Laminariales, which is mainly restricted to the North Pacific do however have a few exceptions to its distribution, one of these is *Laminaria digitata* which is restricted to the Atlantic Ocean (tom Dieck, 1992). Previous studies have examined the temperature ranges for gametogenesis of *L. digitata* (tom Dieck, 1992; Martins *et al.* 2017; Franke *et al*. 2021; Silva *et al.* 2021). 5^{o}C to 12^{o}C are optimal for induction of fertility while temperatures > 17^{o}C inhibit gametogenesis (tom Dieck, 1992; Martins *et al.* 2017). However, slight variations in the optimum range may depend on the location the individuals of the species are collected from. Local adaptation is an ongoing area of question when considering intraspecific differences in the thermal niches between populations (King *et al.* 2018a). Evidence shows local adaptation in response to stress originating from elevated temperatures for *L. digitata* populations which are considered low-dispersal (sessile) species (King *et al.* 2019, 2020), in response to not being able to disperse into future suitable niches (King *et al.* 2018a). Upon increased warming, sessile species such as *L. digitata,* if exhibiting intraspecific differentiation may become locally eradicated (King *et al.* 2018a). Still, local adaptation is important when examining differences within a species and in predicting responses to future climate scenarios. Nonetheless, when considering warming over decades, local adaptation will likely not be possible as the rate of the present-time warming outcompetes the rate of natural selection (King *et al.* 2018a; Jump and Penuelas, 2005).

With rising mean temperatures, extreme temperature events are more likely to occur (Poloczanska *et al.* 2012). Thus, understanding the effects of variations in temperature regimes on the physiological and biochemical responses of a species are important to consider in order to increase further knowledge on the subject (Poloczanska *et al.* 2012; Wernberg *et al.* 2016).

This study examined potential functional differences between two genotypes of the same species, *Laminaria digitata*, when applying different nutrient and temperatures regimes based on local data from two sites, Quiberon and Bodø. In an attempt to determine whether both populations and their respecting life phases are affected by the treatments and how they respond. The study highlights effects on gametogenesis, juvenile sporophyte formation, gametophyte density, and growth under a set of treatments and conditions. Examination of local adaptation for each genotype is considered, in an attempt to provide knowledge on how developmental mechanisms are achieved through suboptimal or optimal nutrient and temperature conditions that can occur naturally in the environment in which the species is present.

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TITLE

Effects of Temperature and Nutrient Regimes on the Recruitment of *Laminaria digitata*

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Keywords: Kelp gametophytes, local adaptation, temperature, nutrients, recruitment

Abstract

Kelps are important macroalgae found in the temperate rocky marine habitats along the northern Atlantic Ocean coast. Kelp forests and its services to the ecosystem are globally at risk due to anthropogenic activity and climate change, by altering the temperature gradient and the nutrient input and where the kelps exist. This could be detrimental for the coming generations of the kelp, *Laminaria digitata,* which is one of the major kelp species in the north Atlantic Ocean. As of this, nutrients and temperature regimes that allow microscopic phases for gametogenesis and survival could be at risk. As gametophyte growth and sporophyte development have different optimal temperatures, the microscopic life phases develop at different rates when exposed to different environmental conditions. To investigate the effects of temperature and nutrient regimes on gametophyte growth, gametogenesis, and early sporophyte formation of *L. digitata*, two populations (Bodø and Quiberon) were exposed to four temperature regimes (4, 8, 12, 18^{o}C) and two nutrient treatments (high (HN) and low(LN)) based on local data from each site. Bodø represents the intermediate zone, which might provide optimum conditions while Quiberon represent the southern distributional limit. The experiment followed a factorial design, testing all temperatures in both nutrient conditions through a mechanistic approach considering both sites. We hypothesized that the response of each population shows local adaptation relating to local nutrient and temperature histories. Results showed that the gametophytes of each population responded differently to the treatments, especially in respect to sporophyte formation, however had similar patterns for development in general. Sporophyte recruitment was 7% higher for the Quiberon population, however, both populations responded positively to optimal temperatures (8 and 12^{o}C) in terms of ontogenetic development. Moreover, both populations interaction with temperature were significantly related to sporophyte recruitment, with Bodø also having a response to the nutrient treatment. In both populations, longer periods of increased temperatures (18^{o}C) enhanced vegetative gametophyte growth but inhibited sporophyte production. This study provides an insight to how *Laminaria digitata* gametophytes responds to forced environmental conditions in terms of survival, growth, and recruitment. It shows distinct differences between populations, exhibiting local adaption based on local nutrient and temperature histories. With emphasis on which treatments results in the optimal conditions to further knowledge on the adaptation responses of the species to environmental conditions and climate change.

1.Introduction

Laminaria digitata is a kelp species belonging to the order Laminariales which extends along the European coast of the north Atlantic Ocean in temperate rocky marine habitats. Kelps do not only provide shelter, food, and resources for multiple variants of marine organisms (Steneck *et al.* 2002), they also present themselves with a high ecological value as marine bioengineers (Martins *et al.* 2019). Additionally, kelps play a large role in structuring the marine habitats that harbors an array of species by protecting the sediments from erosion (Martins *et al.* 2017; Steneck *et al.* 2002). Despite having such a significant impact on the ecosystem, research expenditure on kelps in some areas in the world is minimal (~10%) in comparison to the research done on other and similar ecosystems (Bennett *et al.* 2016).

The life cycle of Laminariales is haplo-diplontic heteromorphic which means that it alternates between two main developmental phases (Figure 1), with a haploid phase as microscopic gametophytes and diploid stage as microscopic sporophytes (Kain, 1979). As the sporophytes become fertile, parts of the blade change into sori which produce haploid meiospores through the process of meiosis. From these meiospores, further development leads to the production of dioicous gametophytes through germination (Martins *et al.* 2017).

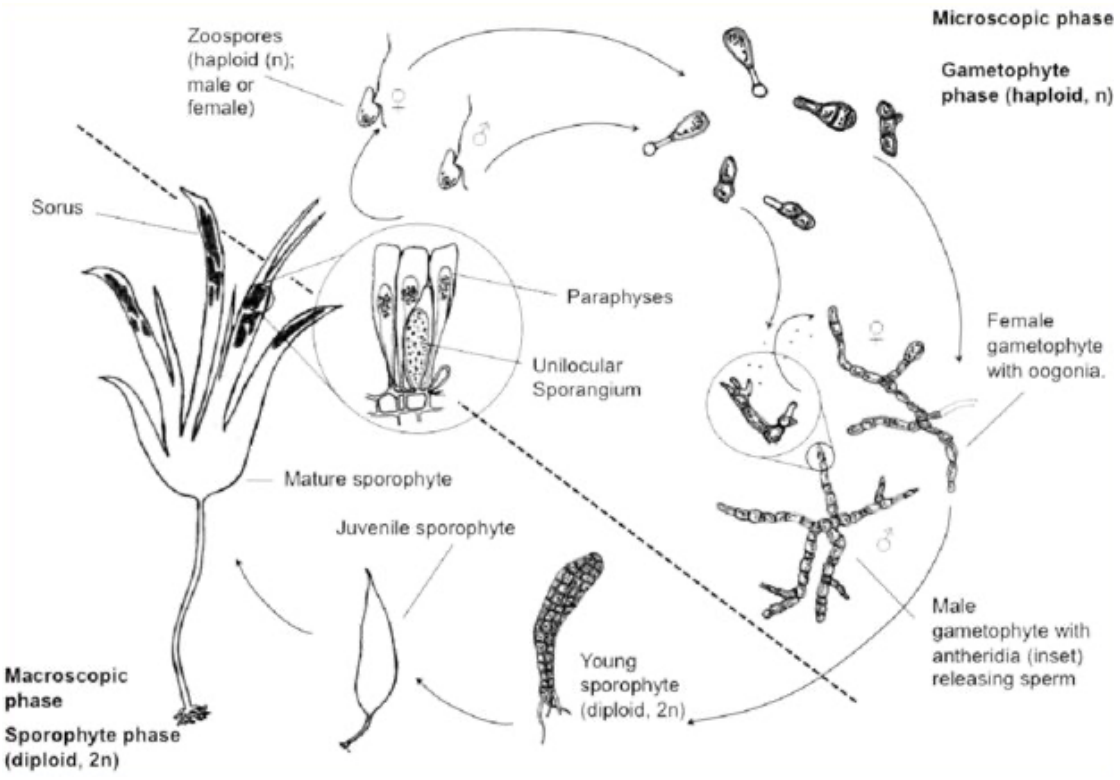

Figure 1: showing the life stages of *Laminaria digitata* (Edwards and Watson, 2015).

Sexual reproduction under the Laminariales is oogamous, meaning that reproduction is explained by the union of a mobile male and immobile female, by the females' eggs releasing the pheromone lamoxirene and attracting the male spermatozoids (Maier and Müller, 1986; Müller *et al*. 1979; Schiel and Foster, 2006). Success of the reproduction leads to development of a microscopic sporophyte (2N), which grows into a macroscopic sporophyte (2N), and the development of a new generation where the life phases continue. A fertile macroscopic sporophyte has the potential in producing millions of spores, setting the respecting generations up for reproductive success if the conditions are favorable (Roleda, 2015).

Another aspect of the kelp's importance is how economically important the species is in terms of providing food and alginate products, with newer applications for a means of clearing contamination through bioremediation in addition to its potential as an alternative to fossil fuel (Bartsch *et al.* 2008). Expanding the knowledge of the potential importance of kelps. Due to the risk of losing the kelp forests and its services to climate change, a continuous effort has been set in motion to study the effects of ocean warming on these ecologically and socioeconomically important marine species (Wernberg *et al.* 2016). *L. digitata* have distinctly different temperature ranges for each developmental phases between gametophyte growth, gametogenesis, and sporophyte recruitment (Martins *et al*. 2017; tom Dieck, 1992), which highlights the importance of studying the effects temperature can have on development of kelp gametophytes. Because the gametophytes are omnipotent, they have an advantage in which they can remain vegetative and halt development through gametogenesis under unfavorable conditions until the environment presents itself with the right conditions (Barradas *et al.* 2011; Bartsch *et al.* 2013; Martins *et al.* 2017). An increase in seawater temperature is therefore likely to delay gametophyte growth and photosynthesis (Roleda, 2015), however, gametophytes that remain vegetative serves the remaining kelp population with a seed bank on the aquatic substrate. As the microscopic stages of *L. digitata* are more resistant to unfavorable conditions, delaying further development may therefore be more favorable as it has the potential to enhance sporophyte recruitment under suitable conditions (Barradas *et al.* 2011; Martins *et al*. 2017).

Due to the early microscopic stages being exposed directly to environment, in contrast with terrestrial plants seed coverings, they reflect characteristics from their belonging environment as well as restrictions due to environmental conditions and availability to nutrients (Morelissen

et al. 2013; Schiel and Foster, 2006). Restrictions include proximity to herbivores, water currents, sediments and are linked to the mortality of macroalgae, affecting the population dynamics through a bottleneck scenario (Lotze *et al*. 2000; Morelissen *et al.* 2013).
With rising mean temperatures, extreme temperature events are more likely to occur. Thus, understanding how variations in temperature regimes alters the physiological and biochemical responses of a species are important to consider in expanding the existing knowledge of these macroalgae (Poloczanska *et al.* 2012; Wernberg *et al.* 2016). Evidence shows local adaptation in response to stress originating from elevated temperatures of *L. digitata* populations, which are considered low-dispersal (sessile) species (King *et al.* 2019, 2020). Upon increased warming, sessile species such as *L. digitata,* if exhibiting intraspecific differentiation, may become locally eradicated as a result of being unable to disperse into future suitable niches (King *et al.* 2018a).

This study examined potential functional differences between two genotypes of the same species, *Laminaria digitata*, when applying different nutrient and temperatures regimes based on local data from two sites, Quiberon and Bodø. In an attempt to determine whether both genotypes and their respecting life phases are affected by the treatments and how they respond. The study highlights effects on gametogenesis, juvenile sporophyte recruitment, gametophyte density, and growth under a set of treatments and conditions. Examination of local adaptation for each genotype is considered, in an attempt to provide knowledge on how developmental mechanisms are achieved through suboptimal or optimal conditions that can occur naturally in the environment in which the species is present.

2. Materials and Methods

2.1 Study sites and their temperature profile

Laminaria digita sporophytes were retrieved from two locations along the European northwest Atlantic coast (Bodø, Norway BOD 67.2904ºN, 14.4049ºE and Quiberon, France QUI 47.4821ºN, 3.1211ºW) representing an area with optimum temperatures and an area at the southern limit with sub-lethal summer temperatures (Figure 2). From each sporophyte, single clonal male and female gametophytes were isolated. The sites examined are both areas which contributes to a larger area which are known to inhabit the species *Laminaria digitata*, considering both optimum and sub-lethal temperatures. Sea Surface temperatures (SST) over the last 10 years on average for the winter and summer were considered (Seatemperature.info). For winter: BOD=4ºC and QUI=9.2 ºC, and for summer: BOD=12ºC and QUI=18.1 ºC.

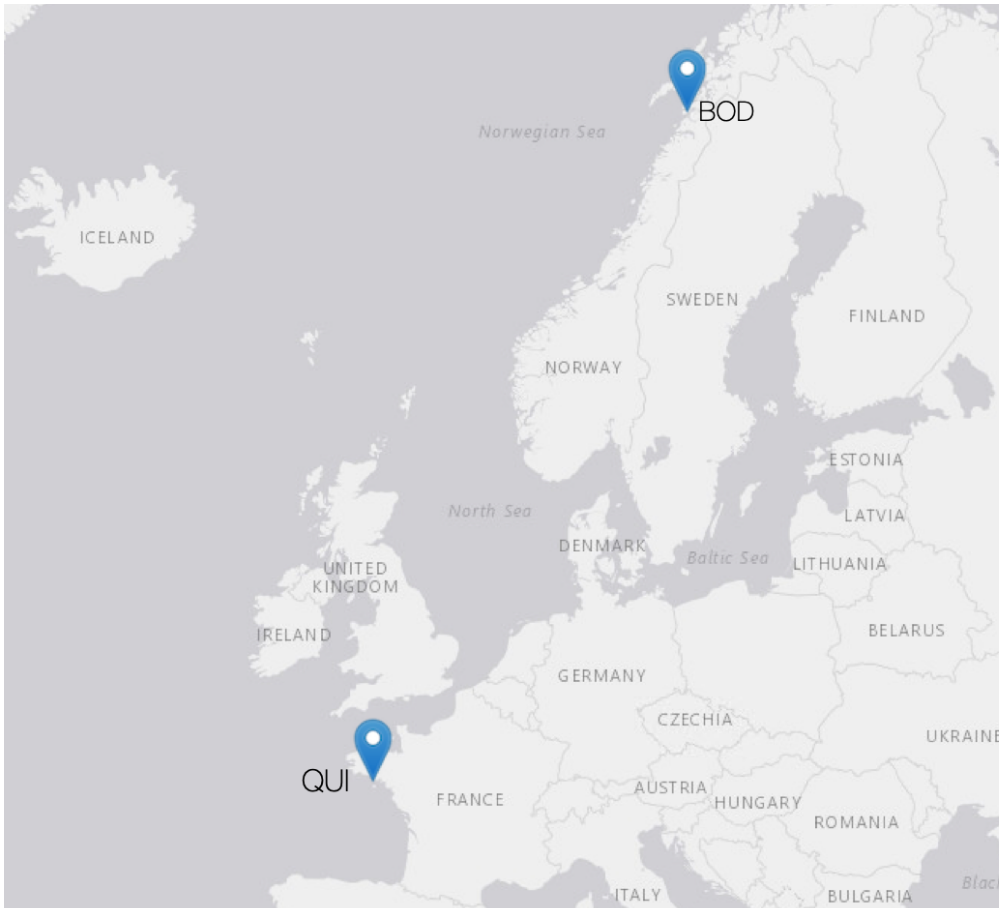

Figure 2: The sampling points along the Northern European coast.
Marked points are Quiberon (QUI) and Bodø (BOD).

The temperature levels considered were derived from the local data for winter and summer averages from each site (Quiberon and Bodø) (Seatemperature.info). The temperatures levels

followed the same pattern for each study site for winter low (4°C), winter intermediate (8°C), summer intermediate (12°C), and summer high (18°C). This allows for examination of local adaptation for the two genotypes used.

2.2 Study material and culture conditions

Fertile *L. digitata* sporophytes were sampled from the infralittoral fringe at each of site (BOD/QUI) and stored in ambient seawater tanks for three days. Spores were retrieved from soral tissue following the protocol of Bartsch (2018). Details are given in Schimpf (2021). At a later stage single male and female vegetative gametophytes were isolated resulting in separate clonal stocks from separate sporophyte individuals per site. These were then deployed into cultivation at 15°C in sterile ½ Provasoli enriched seawater (PES, Provasoli 1968) with 3 µmol photons $m^{-2}s^{-1}$ red LED light controlled by ProFiLux 3 (GHL Advanced Technology, Kaiserslautern, Germany) (Schimpf, 2021). Cultures were changed with ½ PES every 3 months.

2.3 Stock solutions and seeding of gametophytes

Two stock solutions were created from similar amounts of 4 clonal unialgal isolates per site, one with males and one with females. Equal quantities of the clonal isolates gently grinded with a pestle and mortar. Thereafter, the gametophytes were washed through a 100 µm sieve with artificial seawater into two separated beakers which were placed on a stirring plate at 250rpm to keep the gametophyte fragments in suspension. The target density was 600 gametophytes cm^{-2}, which was estimated by placing 0.5 ml and 1 ml of each stock solution into two separate Petri dishes (Ø=6cm) dissolved in 100mL seawater and counting the density at 10x under an inverted microscope (Olympus CKX41, Tokyo, Japan) after settlement. The density was calculated by counting number of fields and gametophytes needed to reach 400 gametophytes. From the density calculated, 40 Petri dishes (Ø=6cm) that were needed for the experimental design, were prepared with the adequate density to reach 600 gametophytes/cm^2 per replicate for the population examined. These steps were repeated for both populations (BOD/QUI).

2.4 Experimental set-up

The focus of the study was to examine the interactive effects of high and low environmental nutrient (N/P) conditions with temperature gradients spanning across the local temperature history of both sites on gametophyte survival, growth, gametogenesis, and juvenile sporophyte

formation of Laminaria digitata. Therefore, we designed the following experimental set-up. The two populations, Bodø and Quiberon, were investigated consecutively for logistic reason but under an otherwise identical set-up and pre-treatment.

Five replicate gametophyte Petri dishes (Ø=6cm) with a target density of 600 gametophytes cm^{-2} filled with 100mL of artificial seawater (AS) at 35 PSU enriched with Nitrate (N) and Phosphate (P) – free von Stosch medium (20mL L^{-1}) plus N/P stock solutions were prepared for each treatment. Two nutrient conditions (high nutrient (HN) and low nutrients (LN)) were tested at four temperatures spanning across the seasonal temperature regime of both populations (4, 8, 12 and 18°C) for each population (5 x 2 nutrients x 4 temperatures = 40 dishes per population). To examine local adaptation and functional differences between the populations.

To utilize the temperatures from each site, a factorial design was applied derived from both sites' annual temperatures. Constant temperatures were achieved in water baths controlled by thermostats (Huber Variostat CC + Pilot ONE, Peter Huber Kältemaschinen GmbH, Offenburg).

Starvation in artificial seawater (Tropic Marin Sea Salt, Tropic Marin, Wartenberg) was issued two weeks prior to experimental start, for the gametophyte isolates to become depleted in nutrients. This was done in order to minimize the amount of nutrients that had already accumulated in the tissue during pre-cultivation in red light and ½ PES.

Table 1: Showing the acclimation procedure from t-5 to t0 for each of the water baths used.

	Acclimation period						*Experimental phase*
Target temperature	Water bath No.	Day -5 (t-5)	Day -4 (t-4)	Day -3 (t-3)	Day -2 (t-2)	Day -1 (t-1)	Day 0 (t0) – Day 20 (t20)
18°C	1	15	15	15	15	16	18
12°C	2	15	15	15	15	14	12
8 °C	3	15	15	14	12	10	8
4 °C	4	15	12	10	8	6	4

Before start of the experiment gametophytes were slowly acclimated to experimental temperatures and allowed for recovery from seeding and grinding stress for the gametophytes under low irradiances (Table 1). The acclimation started 5 days before experimental start (t-5) from 15ºC in 2ºC steps per day, with the final step taking place on day 0 (t0) (Table 1, Figure 2). At the start of the experiment irradiance was increased to 15 ± 1 µmol photons m^{-2} s^{-1} white light. The whole treatment took place in a 16:8h light: dark cycle (LD) under LED lamps (ProFiLux 3; GHL Advanced Technology, Kaiserslautern, Germany).

near darkness (1µM light) using dark netting covering the replicates.

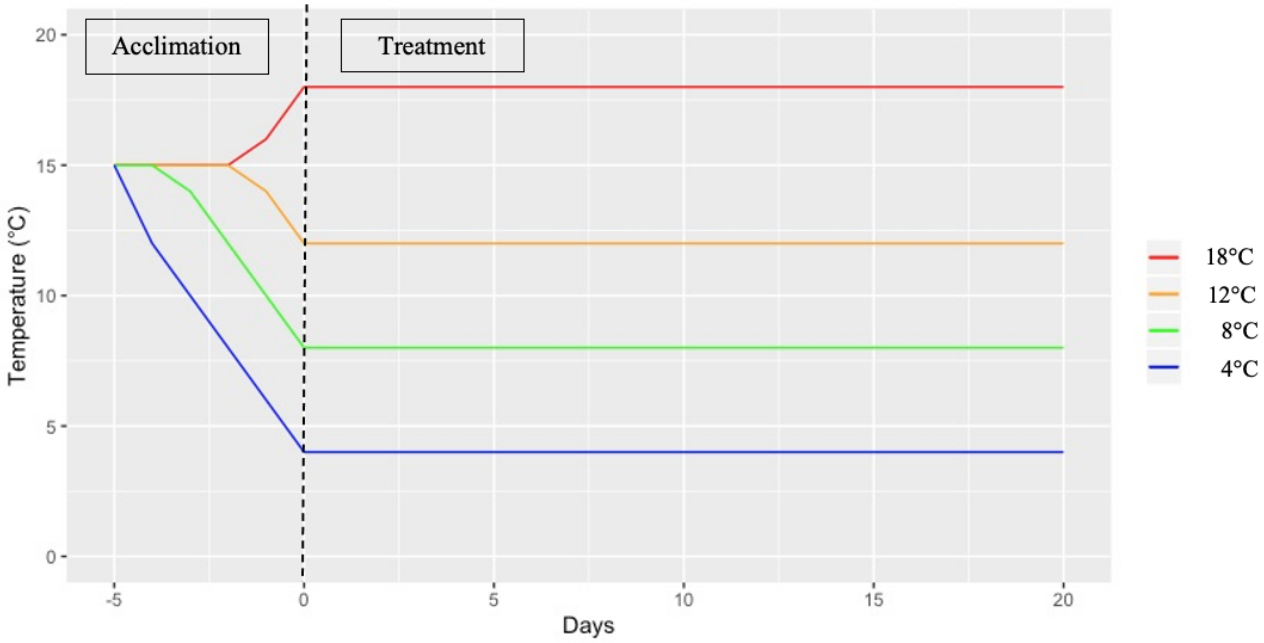

Figure 2: Timeline of the experiment, with acclimation (day -5 – day 0) and treatment (day 0 - day 20) periods indicated. The end of acclimation is separated from the treatment period with a dashed line.

2.5 Cells per gametophyte

At the beginning of the experiment (day 0), the number of cells per gametophyte (male and females) of each population was counted with 20x magnification under an inverted microscope (Olympus CKX41, Tokyo, Japan). Per sex (male/female) for 5 replicates, the number of cells for 10 gametophytes were counted (n=5).

2.6 Nutrient treatment

Once the intended gametophyte density was reached (600 gametophytes/cm^2) and added to the 40 replicate petri dishes (Ø=6cm), the nutrient enrichment medium was added to each of the replicates. The nutrient enrichment medium was a modified autoclaved Von Stosch solution (VSS), which was mixed with artificial seawater (Tropic Marin Sea Salt, Tropic Marin, Wartenberg) to reach the total medium of 100ml per dish. The VSS was created as according to the standard recipe (von Stosch, 1963), but without adding NO_{3-} and PO_4^{3-}, to be able to control the concentrations added. This allowed for a mechanistic approach to follow when adding the different concentrations of NO_{3-} and PO_4^{3-}, which was derived from the winter high and summer low (Ibrahim *et al*. 2014; Wassmann *et al*. 2000; Somlit.fr) from each of the sampling sites (QUI/BOD) (Table 2, Figure S3).

Table 2: Target nutrient concentrations considered for the experimental set-up, based on seasonal variations derived from each sampling site (BOD/QUI). High (HN) and low (LN) nutrient concentrations are equal for both populations.

High Nutrient Conc.		***Low Nutrient conc.***	
(winter)		***(summer)***	
NO_{3-} (µM)	PO_4^{3-} (µM)	NO_{3-} (µM)	PO_4^{3-} (µM)
15	0.5	1	0.5

To be able to separately add $NaNO_{3-}$ and PO_4^{3-}, two nutrient stocks solutions of 0.1M $NaNO_{3-}$ and another of 1mM PO_4^{3-} were prepared to reach the target nutrient concentrations (high/low nutrient) in the artificial seawater that were prepared for the experiment (Table 2).

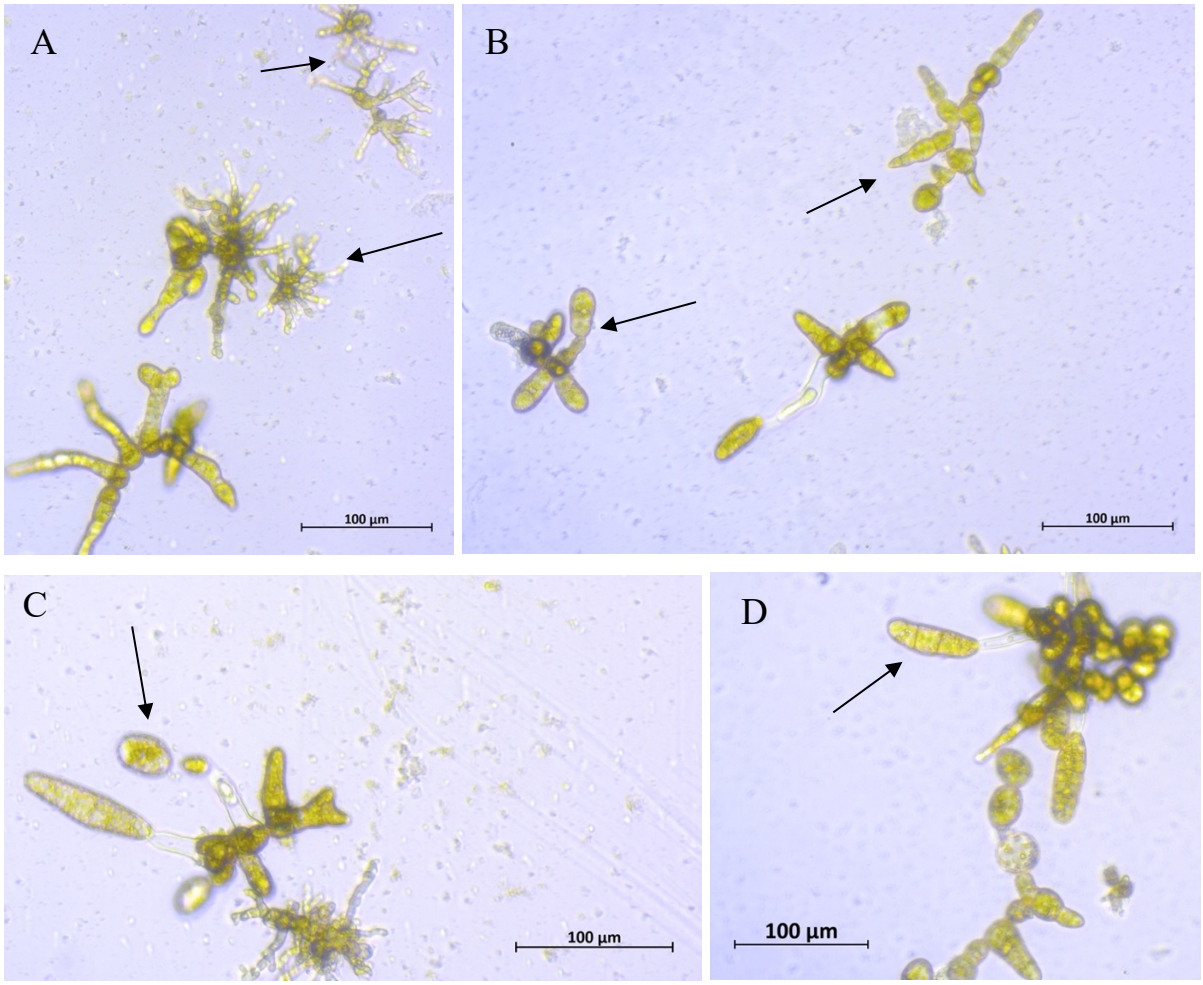

Figure 3: Exhibition of different stages of gametogenesis in Laminaria digitata. Fertile male gametophytes with antheridia (A) and vegetative females (B). Female gametophyte with eggs and a sporophyte (C), a female gametophyte with a sporophyte with clearly visible cell division bands (D). Arrows indicate the gametophytes developmental stage, 10x magnification.

2.7 Quantification of gametophyte density and ontogenetic stages

Number of gametophytes and their ontogenetic stages during gametogenesis were quantified at day 0, 5, 10, 15, and 20 under an inverted microscope (Olympus CKX41, Tokyo, Japan) and with the help of a counter (Assistant 345 counter AC-8, HechtAssistant, Sondheim, Germany). Density was accounted for by counting a total of 400 gametophytes per replicate and respecting grid fields, differencing between males and females at 10x magnification. To study the development of the gametophytes, females were categorized into three ontogenetic stage groups: vegetative (Figure 3b), with eggs (Figure 3c), or with sporophyte/s (Figure 3d).

Females with sporophytes were only considered if a first cell division of the extruded egg was visible and it was attached to the oogonium (Figure 3c and 3d). The class “females with eggs” was considered when the egg was colored and attached to the oogonium (Figure 3c). On day 0

the number of fields required to count ~200 females were estimated and used as a basis for the following counts. Random counting was achieved by moving the Petri dish from the left to the right, avoiding overlaps of grid fields. All gametophytes within the grid and at the outer top and left-side borders were considered. The gametophytes crossing the right-side and bottom of the grid were omitted. On average, 60-70 grids were counted per replicate.

2.8 Gametophyte growth

Gametophyte growth was measured by capturing photographs every 5 days throughout the experiment (t0, t5, t10, t15, and t20) with a camera (AxioCam ERc5s plus ZEN 2 Blue edition 2.3; Zeiss, 2019) mounted to an inverted microscope at 10x magnification. Per replicate, a minimum of 30 gametophytes were recorded. Image analysis of the photos was done by using the image analysis program, FIJI ImageJ (Schindelin *et al.* 2012). During the analysis, the area of each gametophyte (μm, micrometers) in all photos (30) was considered and each measured one by one. Empty spaces within the gametophytes, such as the cells orientation forming circle, were omitted in the analysis. Additionally, all extruded eggs and sporophytes were excluded from the analysis of the area. The data from each replicate at each of the sampling points (t0-t20) were then saved as CSV files for analysis of gametophyte growth and increase in area throughout the experiment.

2.9 Nutrient analysis of seawater

After the gametophytes had settled and acclimation of the replicates was complete (t0), water samples were taken of the stock HN and LN VSS. Additional water samples were taken at t21 from the Petri dishes in order to examine how much nutrients were utilized by the gametophytes in the time period of the experiment (t0-t21). In both instances, 10ml of the medium was sampled with pipettes, placed in falcon tubes (50ml), and analyzed through a continuous flow analyzer (CFA) for quantity of NO_{3-} and PO_4^{3-} in all samples (Grasshoff *et al*. 1999). Per study site, all replicates were analyzed and data from the analysis compared between initial and final nutrient concentrations.

2.10 Statistical analysis

Statistical analysis was performed by using R version 3.6.1 (R Core Team, 2019).

Initially, a Levene's test (Levene, 1960) for homogeneity of variance was done to check that the variances were similar. Shapiro-Wilk normality test (Shapiro and Wilk, 1965) for normal distribution was also used before analysis. In the event of a significant difference ($p<0.05$), a

non-parametric statistical test, such as Kruskal -Wallis ANOVA or Mann-Whitney U-test was used. However, with no significant difference, ANOVA (Analysis of variance) test were assessed. Confidence level used on all tests was 0.95

One-way ANOVA was initiated to test the initial gametophyte densities for both populations, as the Levene's and Shapiro-Wilk's test were not significant ($p>0.05$). Another One-way ANOVA was used to test if cells per gametophyte varied across populations and between sexes. In the instance of a statistically significant difference, the *post hoc* Tukey's test ($p<0.05$) was utilized to determine the differences between treatments, along with pairwise comparisons.

The sex-ratios at t5 were investigated for each population with one-way ANOVAs to check if the ratio (M: F) were the same across all treatments. Additionally, at t20, a two-way ANOVA was used to examine if temperature x nutrients had an interaction on the final sex-ratio for each population.

The interactive effects of nutrients x temperature on the sporophyte recruitment at day 21 was investigated with non-parametric tests for each population. Due to not following normal distribution homogeneity of variance. To analyze the nutrient factor on recruitment, a Mann-Whitney U-test was used as only two independent groups are compared: HN and LN. For the temperature factor, effects on recruitment were tested with Kruskal-Wallis ANOVA.
Change of gametophyte density over time and temperature for each population was investigated with the two-way RM ANOVA. Density at t20 was compared between populations with a three-way ANOVA. Data was normalized according to the density values at t0 for a direct comparison between populations x nutrients x temperatures.
Gametophyte growth was examined for the Bodø population using a two-way ANOVA, to investigate responses between the nutrient and temperature treatment on the gametophyte size (μm^2) at the beginning (t5) and at the end (t20).

For the nutrient data, similarity of N/P content in the nutrient treatments at t0 between both populations in LN and HN were investigated. LN concentrations with one-way ANOVA and HN concentrations with a Kruskal-Wallis due not following normal distribution.
Additionally, the difference in N/P content in the water samples at t21 between both populations was examined. The LN and HN treatment along the temperature gradient was examined with Kruskal-Wallis tests, for differences between populations and temperatures.

3. Results

3.1 Initial density and cells per gametophyte

The initial density and cells per gametophyte at the start of the experiment (t0) were examined to determine potential differences between populations and sexes. This was implemented to record potential differences between populations as a result of the seeding procedure.

Table 3: Mean cells per gametophyte for Quiberon (QUI) and Bodø (BOD) at day -5 (mean ± SD, n=5).

Mean cells / gametophyte			
QUI		**BOD**	
Male	Female	Male	Female
6.8 ± 0.89	6.1 ± 0.66	7.4 ± 1.75	5 ± 0.4

A one-way ANOVA was executed to examine if the density of gametophytes at the start of the experiment at t0 was equal for all treatments in both populations. The ANOVA tests indicated that not all treatments had the same number of gametophytes a t0, for Bodø ($p<0.05$) and Quiberon ($p<0.0005$). A Tukey contrast test was followed to establish which treatments varied. In the Bodø population, Tukey test revealed that most treatments had similar numbers of gametophytes ($p>0.05$), except for BOD-LN-18 (638 ± 17) that was significantly higher than BOD-HN-18 ($p=0.0233$) and BOD-HN-4 ($p=0.0313$). The same was true for the Quiberon population, where the only treatments that were significantly different from each other were QUI-LN-12 and HN-4 ($p<0.005$), LN-18 and HN-4 ($p=0.016$), LN-4 and LN-12 ($p=0.0179$), LN-8 and LN-12 ($p<0.005$), as well as LN-8 and LN-18 ($p=0.005$). A one sample t-test confirmed that each of the populations had different initial densities at t0 ($p<0.005$).

Additionally, a one-ANOVA was carried out to check if the number of cells per gametophyte was different between populations (BOD/QUI) for both sexes, followed by a Tukey contrast test. Between populations (BOD/QUI), mean cells per gametophyte (male and female) were not significant ($p>0.05$). However, there was a significant difference between Bodø males and females ($p=0.011$) in terms of cells per gametophyte (Table 3).

3.2 Gametophyte density and sex-ratio

3.2.1 Density

Gametophyte density was recorded throughout the experiment and examined between populations to investigate potential differences with interactions of temperature and nutrient treatments. Detailed data is given in Table 4.

Absolute number of gametophytes varied across populations and treatments. At 18ºC gametophyte numbers significantly decreased at day 20 (Table 4).

Table 4: Absolute number of male and female *Laminaria digitata* gametophytes of two populations (Quiberon and Bodø) under high (HN) and low nutrient (LN) treatments and four temperatures over 20 days (mean ± standard deviation, n=5).

	Quiberon		Bodø	
4°C	**HN Treatment**	**LN Treatment**	**HN Treatment**	**LN Treatment**
Time	**No. gametophytes cm²**		**No. gametophytes cm²**	
t0	616 ± 13	610 ± 22	561 ± 24	584 ± 47
t5	668 ± 17	694 ± 36	583 ± 70	621 ± 57
t10	565 ± 43	693 ± 57	605 ± 11	633 ± 133
t15	660 ± 54	731 ± 73	590 ± 41	660 ± 118
t20	669 ± 55	680 ± 63	544 ± 57	618 ± 161
8°C				
Time				
t0	603 ± 15	623 ± 24	592 ± 51	608 ± 32
t5	618 ± 38	655 ± 53	561 ± 52	597 ± 42
t10	593 ± 30	665 ± 18	569 ± 38	619 ± 36
t15	604 ± 42	712 ± 58	565 ± 29	582 ± 36
t20	626 ± 61	662 ± 81	559 ± 39	624 ± 71
12°C				
Time				
t0	584 ± 21	557 ± 16	574 ± 48	574 ± 15
t5	708 ± 58	633 ± 87	642 ± 131	608 ± 66
t10	612 ± 34	597 ± 67	664 ± 172	614 ± 22
t15	657 ± 37	613 ± 80	589 ± 122	629 ± 75
t20	597 ± 48	569 ± 29	629 ± 109	599 ± 65
18°C				
Time				
t0	603 ± 40	563 ± 20	559 ± 27	638 ± 17
t5	616 ± 104	660 ± 89	530 ± 19	567 ± 63
t10	622 ± 41	569 ± 86	531 ± 62	622 ± 91
t15	658 ± 44	643 ± 53	505 ± 60	520 ± 85
t20	527 ± 60	451 ± 55	497 ± 52	596 ± 40

Gametophyte density over time and temperature was investigated using two-way RM ANOVAs for each population. Gametophyte density varied over time and temperature for both populations (Table 5). Moreover, higher temperatures and time lead to a decrease in density of

gametophytes at suboptimal temperatures for most treatments (Table 4 and 5).

Table 5: Results of two RM ANOVA to investigate the *Laminaria digitata* gametophyte density over time (t0-t20) and temperature (4, 8, 12, and 18°C) for Quiberon and Bodø.

	Quiberon			Density	Bodø			Density
Parameter	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
Temperature	3	36	5.7	**0.0028****	3	36	3.58	**0.023***
Time	4	33	12.8	**<0.0001*****	4	33	2.94	**0.0347***
Temperature x Time	12	105	3	**0.001****	12	105	2.39	**0.0091****

Statistically significant values ($p<0.05$) are highlighted in bold. numDF, numerator degrees of freedom; denDf, denominator degrees of freedom.

Additionally, gametophyte density between populations at t20 were investigated with a three-way ANOVA for effects of temperature x populations x nutrients. As t0 density data of both populations were different, density t20 values were normalized according to t0 densities for a direct comparison.

Table 6: Three-way ANOVA examining the effects of nutrients x temperature x population on the *Laminaria digitata* gametophyte density (cm^{-2}) at the end of the experiment at day 20.

Parameter	numDF	denDF	F-value	Density p-value
Nutrient	1	64	1.37	0.2446
Population	1	64	0.81	0.3708
Temperature	3	64	9.61	**<0.0001 *** **
Nutrient x Population	1	64	4.21	**0.0442 ***
Nutrient x Temperature	3	64	1.24	0.3011
Population x Temperature	3	64	4.79	**0.0045 ****
Nutrient x Population x Temperature	3	64	1.44	0.2381

Statistically significant values ($p<0.05$) are highlighted in bold. numDF, numerator degrees of freedom; denDf, denominator degrees of freedom.

While temperature had an overall significant effect on gametophyte density at the end of the experiment, there were also significant Nutrient x Population and Temperature x Population effects (Table 6). Results (Table 6) showed that gametophyte density at t21 had a significant interaction with temperature ($p<0.005$) alone, as density was not affected by nutrient treatments ($p>0.05$) (Table 6). There was a significant interaction between nutrients x population ($p=0.0442$) and population x temperature ($p=0.0045$) on gametophyte density at day 20, indicating that gametophytes were influences differently by the temperature regimes and nutrient concentrations between populations.

3.2.2 Gametophyte sex-ratio

Gametophyte sex-ratio (male: female, M:F) was investigated at t5 and at t20 to examine whether temperature and nutrient treatments had a differential effect on the survival of the different sexes over time.

As the sex ratio between BOD and QUI was different from the beginning (t5), a direct comparison cannot be made between populations. Unfortunately, the target sex ratio of 1 (M:F) had not been achieved despite sowing from separate male and female stock solutions with known densities. In Quiberon there was approximately 20% more males than females at day 5 (sex ratio of 1.2 (M:F)), while in Bodø there were approx. 30% more females than males (sex ratio of 0.7 (M:F)) (Figure 4). Initial sex-ratio between treatments at t5 was tested to examine if there were any significant difference between the sex-ratio across all treatments for each population. For both populations, a one-way ANOVA was used, showing no significant difference in sex-ratio between treatments ($p>0.05$). Within each population there was no sex-ratio variations among treatments at day 5.

At t20, separate two-way ANOVAs per population were performed to examine how temperature and nutrients affected the sex-ratio, i.e., the differential survival capacity of both sexes at the end of the experiment.

There was no significant interaction between temperature x nutrients and no effect of single factors on the sex-ratio (M:F) for Bodø and Quiberon at day 20. None of the populations were significantly affected, thus both populations responded in the same way. Differences can be seen between populations, which was expected, as the sex-ratio at day 5 already was different, with the Quiberon population having a higher male to female sex-ratio than the Bodø population (Figure 4).

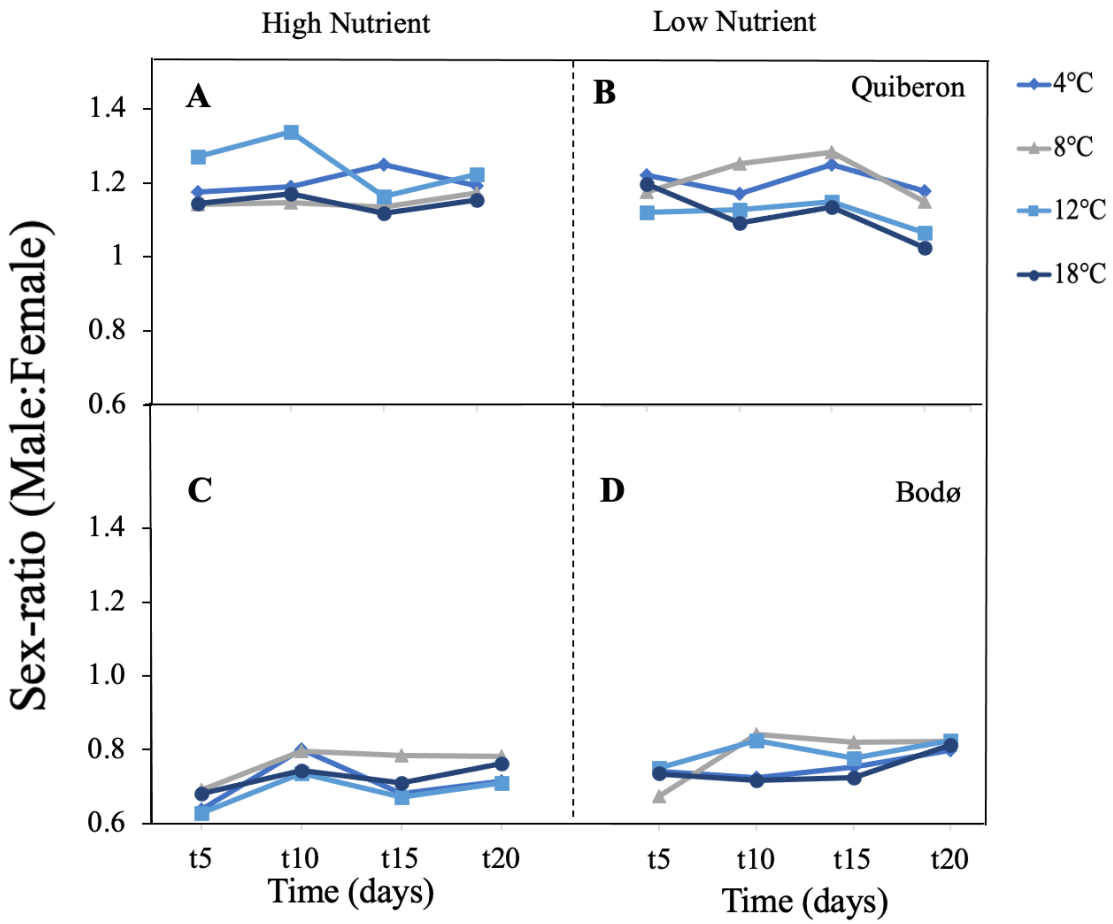

Figure 4: Sex-ratio in gametophytes of *Laminaria digitata* from Quiberon (A, B) and Bodø (C, D) under different temperatures (4, 8, 12, and 18°C) and high (A, C) and low (B, D) nutrient treatments over 20 days (mean values, n=5, SD not shown for clarity).

3.3 Gametogenesis

Time course of ontogenetic development under high and low nutrient conditions as well as the temperature gradient ranging between 4 and 18°C is exhibited in Figure 5. It becomes visible that all gametophytes from both populations only exhibited a low fertility irrespective of temperature and nutrient conditions, not surpassing 20% of gametophytes with eggs or sporophytes (Figure 5). This was even more pronounced with respect to sporophyte formation that stayed very low over 20 days, never surpassing 7%. The 7% sporophyte recruitment was achieved for the Quiberon population at 12°C with high nutrient treatment. The highest sporophyte recruitment for the Bodø population was achieved at the same temperature and nutrient treatment but with only 2% recruitment (Figure 5).

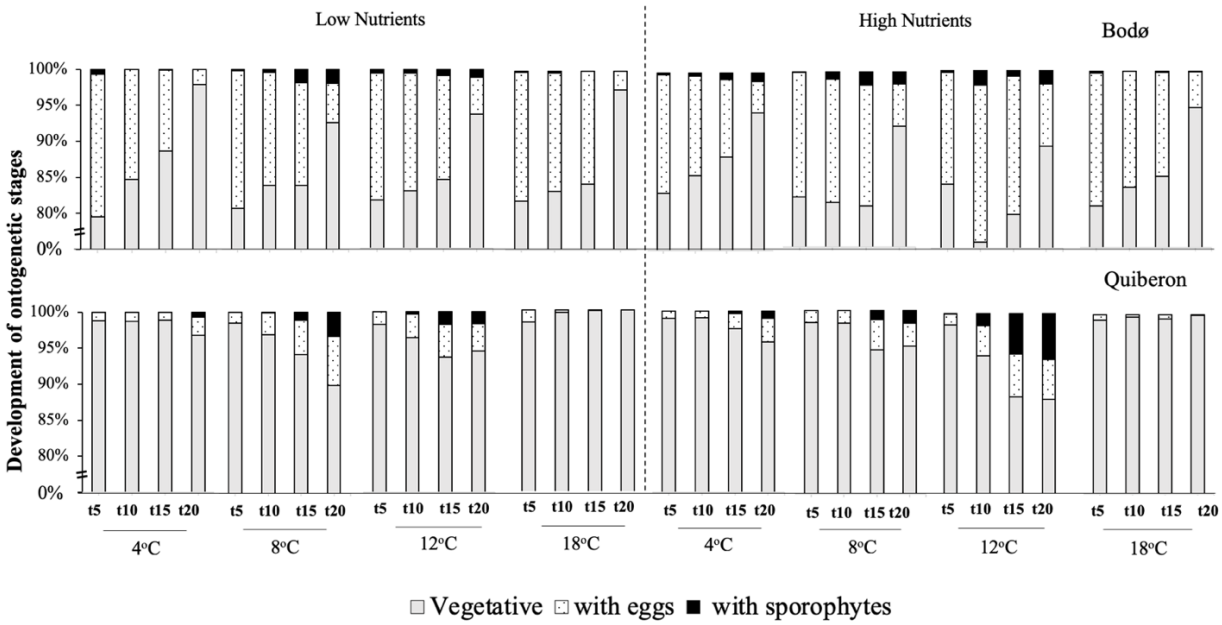

Figure 5: Ontogenetic development of Laminaria digitata female gametophytes from Bodø (A) and Quiberon (B) under a set of nutrient and temperature conditions over time. Female gametophytes were classified in three classes as vegetative, with eggs, or with developing embryonic sporophytes. Counting was done every 5 days over a 20-day period. Data are mean values (n=5), SD are not presented for a clearer visuality.

In terms of the production of sporophytes throughout the experiment, some distinctions can be made when comparing nutrient and temperature treatments. Overall, more sporophytes were produced for each population at 8 and 12°C coupled with high nutrient treatments. The Quiberon population had an increase in the sporophyte to egg ratio throughout the experiment, indicating that the eggs were fertilized. The Bodø population also formed sporophytes under high nutrient treatments at low temperatures (4°C) at t15 and t20 but not under low nutrient concentrations (Figure 5). Few to no sporophytes developed at 18°C in both populations, and gametophytes with egg formation were even much less pronounced in the Quiberon population than in Bodø as in general. In the Bodø population there was a high development of females with eggs (~20%) already at t5 for most treatments, but this did not continue over time. In contrast there was a decrease of gametophytes with released eggs from t5 onwards for the majority of the treatments of the Bodø population (Figure 5).

3.4 Sporophyte formation

In addition to the relative development of the ontogenetic stages, the absolute number of juvenile sporophytes at the end of the experiment on day 21 was quantified (Figure 6) and evaluated based on statistical testing between populations. Thus, to investigate the effects of

nutrients and temperature on the sporophyte recruitment, non-parametric test was used . For Quiberon, the effects of temperature on the recruitment at t21 through the Kruskal-Wallis ANOVA revealed a significant effect (p=0.039) . Additionally, it showed that more sporophytes were recruited at the temperatures 8 and 12 ºC (>18 ºC=4 ºC). However, there was no significant effect of nutrient treatments (p=0.558) on sporophyte recruitment

The Kruskal-Wallis ANOVA proved that for Quiberon there was a significant response ($p<0.05$) between the temperature and the sporophyte recruitment, thus nutrient treatment had no effect on the sporophyte recruitment at t21. In Figure 6, variations in recruitment of sporophytes seem to be distinguished by if the replicates were growing under temperatures of 8 and 12ºC or 4 and 18 ºC.

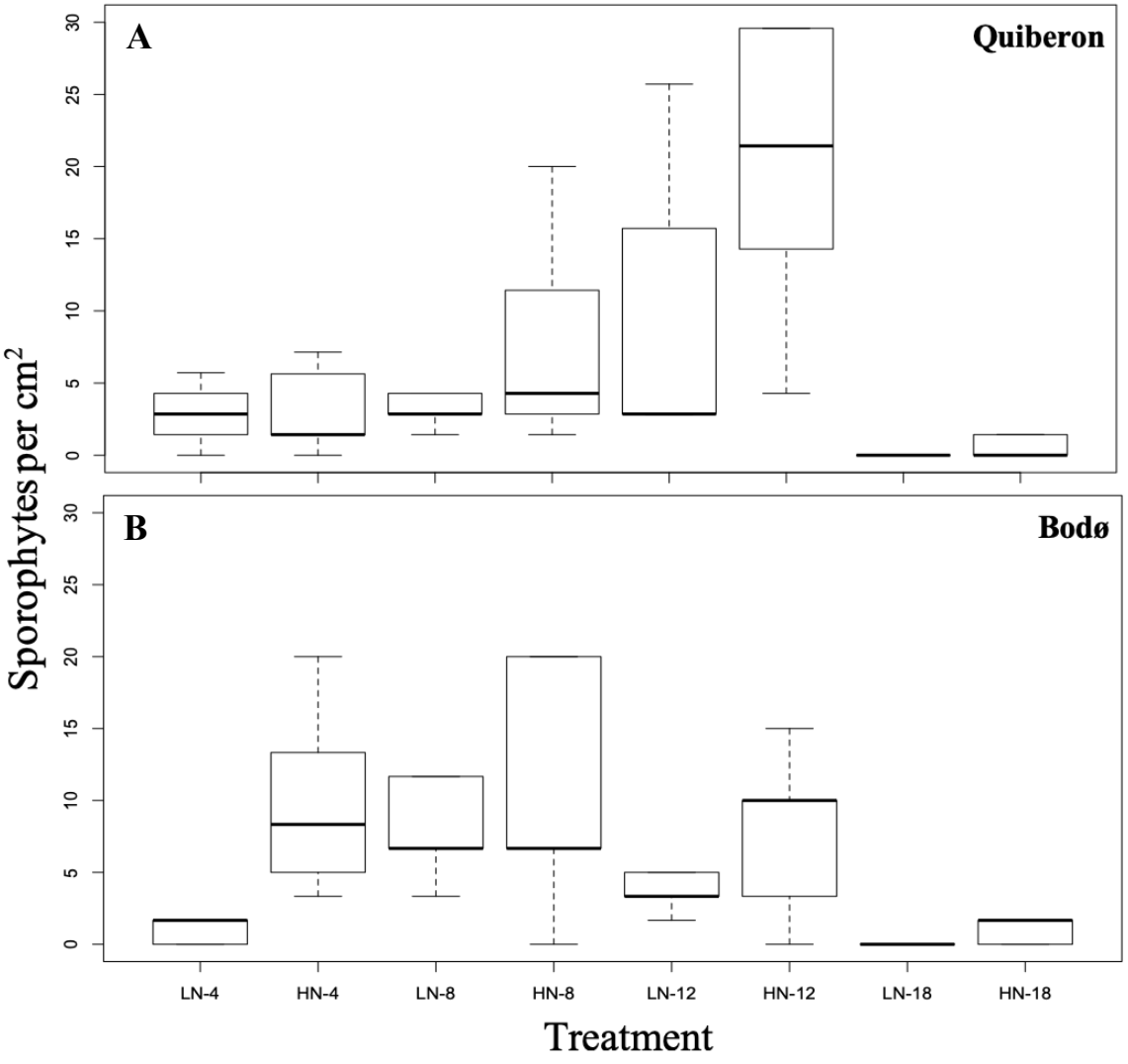

Figure 6: Absolute number of sporophytes (cm^{-2}) formed in two populations of *Laminaria digitata*, Quiberon (A) and Bodø (B), under high (HN) and low nutrient (LN) conditions and temperature treatments (4, 8, 12, 18ºC) at day 21 (n=5). Box plots show median (thick line),

± standard deviation (whiskers), interquartile range (IQR) (boxes).

As seen in Figure 6, variations in sporophytes density are higher for the Bodø population (B) as indicated by higher spread in the standard deviation areas across all treatments than for Quiberon. However, as with Quiberon (Figure 6A), a smaller number of sporophytes are observed at 4 and 18°C, indicating a relationship to temperature. A two-way ANOVA was therefore used to examine if the sporophyte recruitment for the Bodø population was affected by the nutrient and temperature treatments.

A Kruskal-Wallis ANOVA test revealed that there was a significant response between treatments and sporophyte recruitment. With both temperature ($p=0.039$) and nutrient treatment ($p=0.0049$) having an effect on the sporophyte recruitment. For Bodø, temperature and nutrients are shown to be important factors when considering sporophyte recruitment. With more sporophytes developing at 8 and 12°C. Highest sporophyte recruitment per population was observed at QUI-HN-12 with 37 ± 44 (mean ± SD) and BOD-HN-8 with 11 ± 9 (mean ± SD) sporophytes cm^2. As the two populations are affected differently by the temperature and nutrient treatments, they are exhibiting functional differences based on their local temperature and nutrient regimes.

3.5 Gametophyte growth

Gametophyte growth was examined through analysis of gametophytes photos taken at day 0 to day 20. Data from the analysis was given in micrometers squared (μm^2) for the gametophyte area and examined between treatments for the Bodø population.

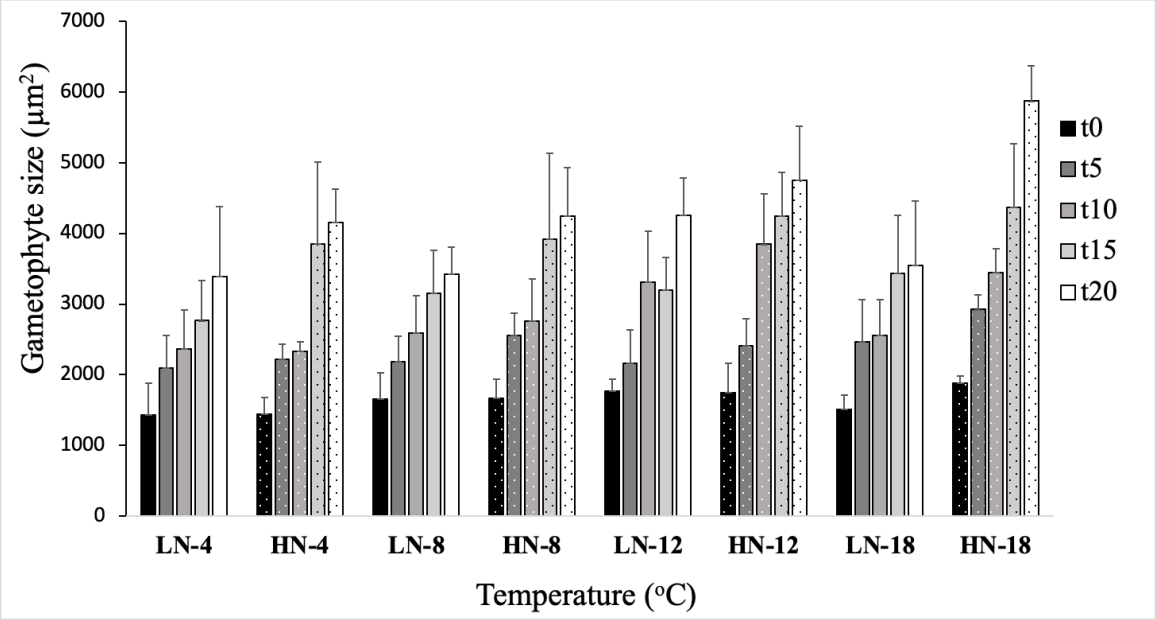

Figure 7: Increase of *Laminaria digitata* gametophytes (μm^2) across 20 days (t0-t20) under different nutrient (LN/HN) and temperature (4, 8, 12, and 18°C) treatments for the Bodø population (mean ± standard deviation, n=5). HN treatments (dots) and LN treatments (no dots), different colors represent time.

Two-way ANOVAS were utilized at t0 and t20 to investigate differences in size for the population (BOD) under treatments (temperature x nutrients). There was no significant interaction of temperature and nutrients on the size of gametophytes at t0. At t20, both temperature ($p=0.01$) and nutrient ($p<0.005$) treatments had a significant effect on gametophyte size. The largest gametophytes developed in HN treatments and at 12 and 18 °C. Results for gametophyte growth shows that from t15-t20, replicates under high nutrient treatments had larger gametophytes than the replicates under low nutrient treatments (Figure 7). More interestingly, the gametophytes at 18°C in HN treatment were larger than the respective LN treatment. With some gametophytes starting to deteriorate at 18°C between t15-20, other gametophytes were still growing in size despite not developing sporophytes. Overall, gametophytes became larger with increasing temperatures. However, an increase in gametophyte size is seen between all treatments across time, with larger gametophytes in HN treatments towards the end (t15-20).

3.6 Nutrients in seawater

3.6.1 Nitrate and phosphate concentrations of artificial seawater and low and high nutrient seawater stock solutions

The nutrient concentrations of nitrate (N) and phosphate (P) in artificial seawater in the initial stock solutions of the high and low nutrient seawater (HN, LN), the N and P concentration in artificial seawater without nutrient addition and the N and P concentrations of all treatments at the end of the experiment (t21) was determined (Figure 8 and 9). Single HN and LN were used for comparison of the treatment nutrient conditions at t0 against which the usage of nutrients by gametophytes was by t21. This allows to estimate how much of the nutrients provided was absorbed by the gametophytes during the treatments. For both populations the initial PO_4^{3-} concentrations were the same, as lower concentrations were unable to obtain in situ, due to natural concentrations already present in the artificial seawater (AS). AS was analyzed and even though no nutrients were added, it contained ~0.5-0.6 µmol L-1 of PO_4^{3-} , thus making lower concentrations unable to obtain.

The initially HN and LN nitrate and phosphate concentrations in seawater were checked between populations in order to verify whether the nutrient environment for the two consecutive experiments were the same. A one-way ANOVA showed that the NO_{3-} content of the LN stock solutions for both populations were the same ($p>0.05$), however differed for PO_4^{3-} ($p<0.05$).

For HN, a Kruskal-Wallis test showed a significant difference for both, the NO3- (X^2=3.86, p=0.0495, df=1) and PO_4^{3-} (X^2=3.97, p=0.0463, df=1) concentration in the stock solution of both populations. However, variations were still low (Figure 8 and 9). In terms of the differences between nutrient content at t21 between populations as a result of the temperature regimes, there were significant differences between temperatures and populations, these were examined through a Kruskal-Wallis test and are described below.

3.6.2 Phosphate

Initial concentrations of AS (artificial seawater), LN and HN served as controls and were compared with final concentrations of seawater of all treatments (Figure 8). Treatments showed a difference in how much of the nutrient medium that was utilized for both populations, with variations between treatments.

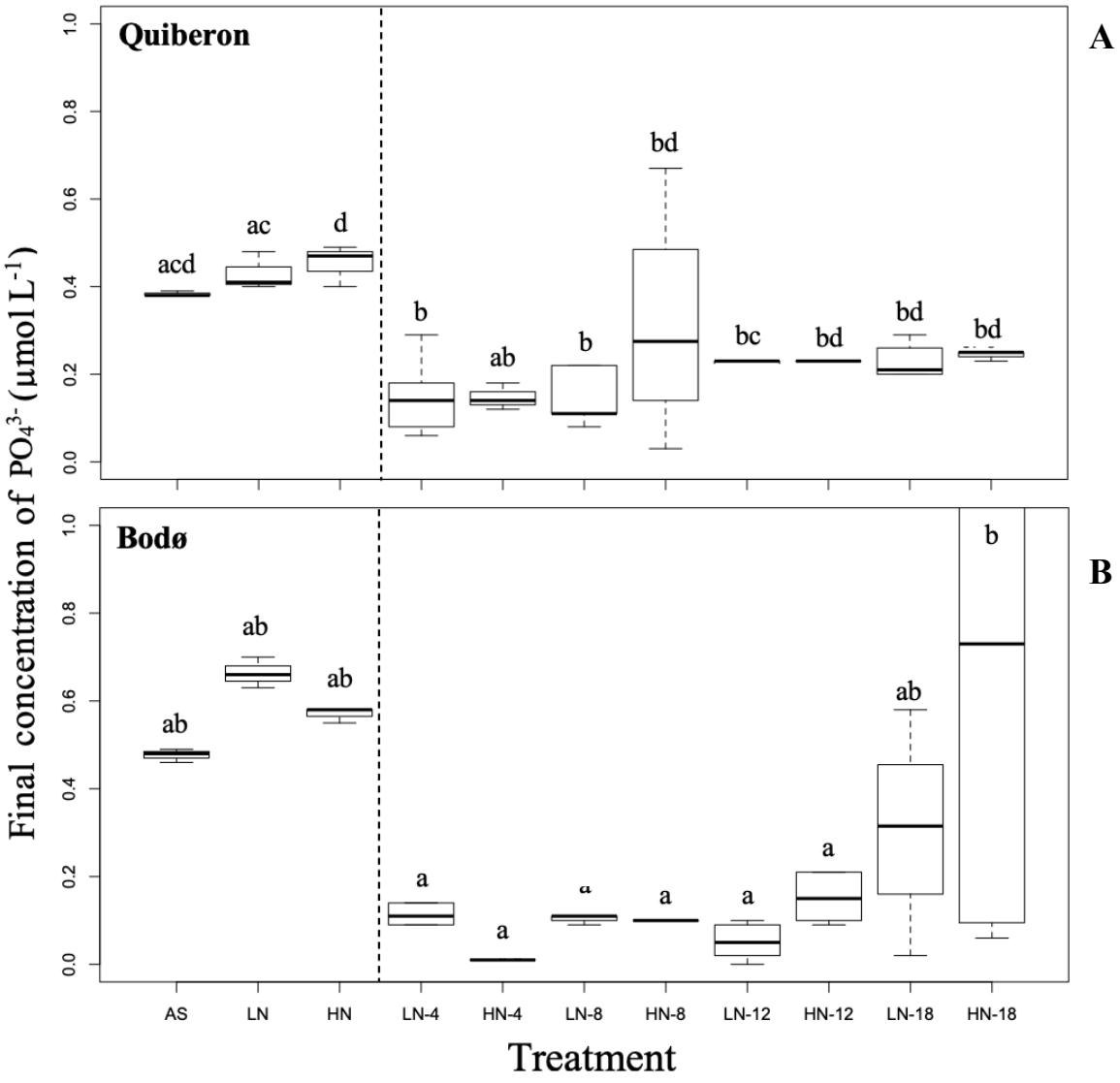

Figure 8: Nutrient concentrations (µmol L-1) for PO_4^{3-} of *Laminaria digitata* gametophytes medium of two populations Quiberon (A) and Bodø (B) under high (HN) and low nutrient (LN) and temperature (4, 8, 12, 18ºC) treatments at day 21. Initial high and low nutrient concentrations (control) indicated by single HN or LN, artificial seawater (AS) concentrations shown for reference (n=5). Box plots show median (thick line), ± standard deviation (whiskers), interquartile range (IQR) (boxes). Initial nutrient concentrations and treatments separated by a dashed line. Letters indicate significant differences.

With respect to nutrient utilization, for both populations, phosphate in the medium at t21 was generally lower than the initial (LN/HN) nutrient concentrations. Which indicates that the nutrients were utilized for growth and development. For the Quiberon population (Figure 8A), phosphate did not have a significant effect on the recruitment of juvenile sporophytes ($p>0.05$). For the Bodø population (Figure 8B), variances within treatments are lower which indicates that the responded replicates responded in a similar manner. An extreme outlier within the Bodø population can be observed in Figure 8B, with treatment BOD-HN-18.

HN phosphate concentrations at t21 were significantly affected by temperature in Bodø and Quiberon (Kruskal-Wallis ANOVA: X^2=20.3, p<0.05, df=7) with highest P at 18°C (18°C > 4-12 °C) with 0.92 µmol L-1 and lowest at 4°C (4°C < 8-12°C) with 0.01 µmol L-1 both in the Bodø population.

For LN (low nutrient), phosphate concentrations at t21 were also significantly affected by temperature in Bodø and Quiberon (Kruskal-Wallis ANOVA: X^2= 17.6, p=0.014, df=7) with highest P at 18°C (18°C > 4-12°C) at 0.23 µmol L-1 in Bodø and lowest in Bodø (12°C < 4, 8, 18°C) at 0.05 µmol L-1 after low nutrient treatment.

3.6.3 Nitrate

As with phosphate, the nitrate concentrations were examined in the beginning of the experiment (t0) in addition to at the end of the experiment (t21), to investigate the final nutrient concentration in the seawater. In terms of how the nitrate (NO_{3-}) concentrations varied between initial and final concentrations, there are more distinct differences between populations and treatments (Figure 8). As initial nutrient treatments varied depending on if the replicates were given high (HN) or low (LN) concentrations, the nitrate utilization was also different. For both populations, LN treatments with initial low concentration of nitrates (1 µmol L-1), was completely depleted by the end of the experiment at day 21. The exception was BOD-LN-18 (Figure 9B), which had about half of the nitrates left (0.57 µmol L-1).

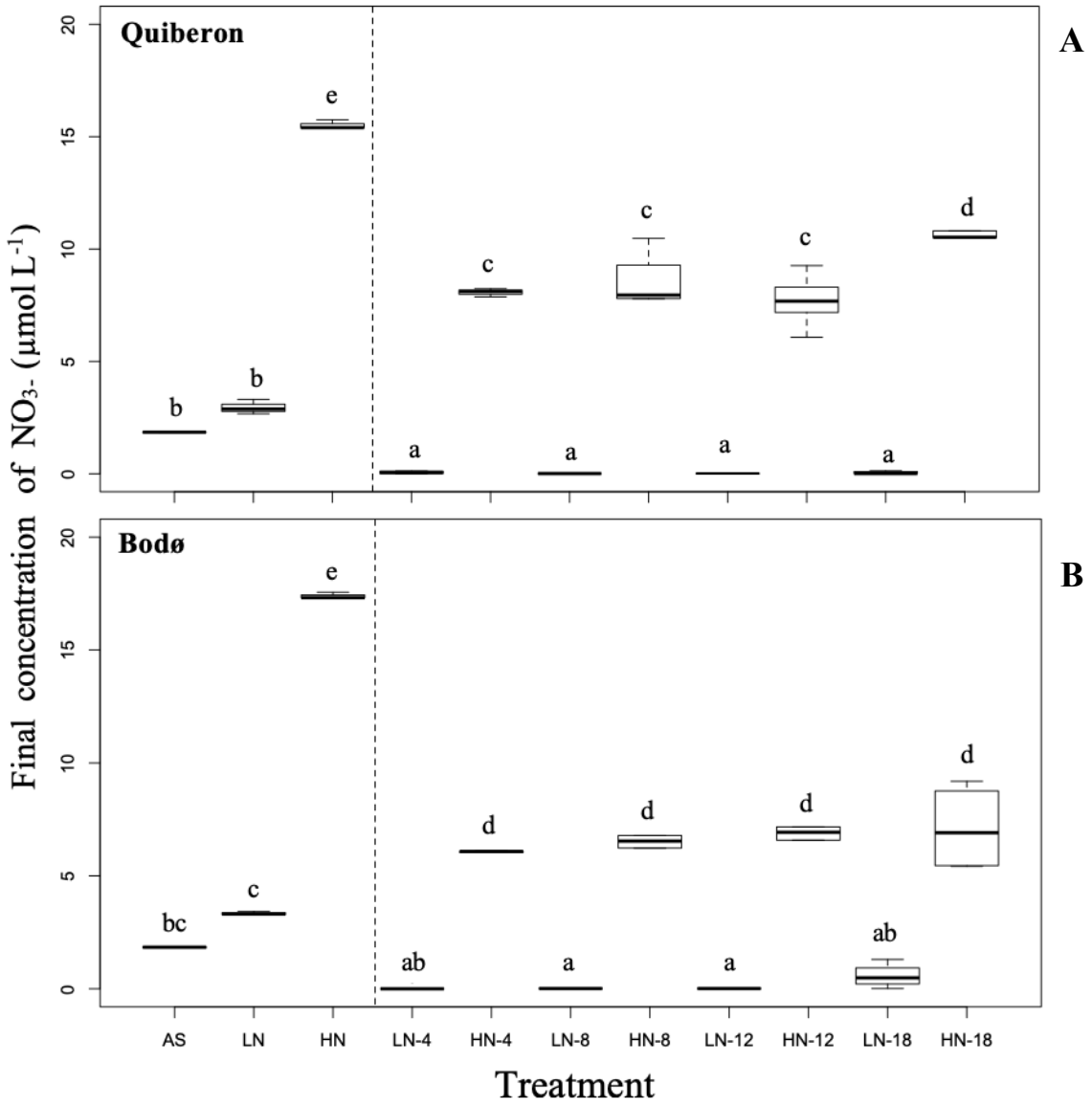

Figure 9: Nutrient concentrations (µmol L^{-1}) for NO_{3-} of *Laminaria digitata* gametophytes medium of two populations, Quiberon (A) and Bodø (B) under high (HN) and low nutrient (LN) and temperature (4, 8, 12, 18ºC) treatments at day 21. Initial high and low nutrient concentrations (control) indicated by single HN or LN (n=5), Artificial seawater (AS) concentrations shown for reference. Box plots show median (thick line), ± standard deviation (whiskers), interquartile range (IQR) (boxes). Initial nutrient concentrations and treatments separated by a dashed line. Letters indicate significant differences.

As exhibited in Figure 9, replicates receiving high nutrient treatments did not fully utilize all the nutrients given to them for both populations (QUI/BOD). Nitrate concentrations in AS was measured at 1.84 µmol L-1 for both Bodø and Quiberon.

For Quiberon (Figure 9A) that is reasonable, as nutrient treatments and sporophyte development had no significant interaction ($p>0.05$). The nitrate concentration at t21 was significantly reduced in all treatments in both populations, by 50-100 % in the LN situation,

by 25-50% in the HN situation. However, as there was a significant interaction between the sporophytes recruited and nutrient treatments for Bodø, more nutrients should have been utilized as final development was still low (Figure 6B). HN nitrate concentrations at t21 were significantly affected by temperature in Bodø and Quiberon (Kruskal-Wallis ANOVA: X^2=21.2, p<0.005, df=7) with highest N at 18°C (18°C > 12-4 °C) in Quiberon while this was the opposite for Bodø (4°C < 8-12°C). Highest value was 10.8 µmol L-1 (QUI-HN-18) and lowest at 6.5 µmol L-1 (BOD-HN-4).

For LN (low nutrient), nitrate concentrations at t21 were also significantly affected by temperature in Bodø and Quiberon (Kruskal-Wallis ANOVA: X^2=16.47, p=0.021, df=7) with highest N at 18°C (18°C > 4-12°C) at 0.58 µmol L-1 in Bodø and lowest also in Bodø (4°C < 8-12°C) at 0 µmol L-1.

4. Discussion

This study highlights the effects of temperature and nutrient regimes on gametogenesis and recruitment of juvenile sporophytes of one of the key kelp species, *Laminaria digitata*, from North-Atlantic rocky coastal habitats. Results showed that the populations (QUI/BOD) show slight local adaption in terms of the response each population had to the temperature treatments. However, the same response range was recorded in both populations. In terms of sporophyte recruitment, gametophytes from Quiberon had an optimum temperature range between 8-12^{o}C, while for Bodø it was between 4-8^{o}C. Which indicates partial local adaptation and histories by each of the sites that the gametophytes were retrieved from.

4.1 Gametogenesis

Overall, there were very low and slow rates of gametogenesis resulting in very low sporophyte recruitment. Additionally, it became obvious that initial high rates of egg production did not translate into sporophytes suggesting problems with the fertilization process itself. Despite this there is an optimum range at 8- 12°C that matches previous studies (Martins *et al.* 2017; tom Dieck, 1992). Although development through gametogenesis was low (minimum 75% remaining vegetative), the optimum temperature range for development was at 8 and 12^{o}C which match previous studies with emphasis on the better temperature range to achieve gametogenesis (Martins *et al.* 2017; tom Dieck, 1992). Considering the Bodø population, a reverse trend with percentage eggs decreasing from t5-t20, can be observed for both high and low nutrient treatments, in comparison to Martins *et al*. (2017).

In Quiberon material egg production only slowly increases across the experimental time period (t5-t20) at 4-12°C, but not 18°C, which is similar to previous studies (Martins et al. 2017, 2020). This translates into a slowly increasing number of sporophytes until t15 but where it stops, for reasons not accounted for. In the Bodø population this situation is much more pronounced already starting day 5, which may be due to ontogenetic development starting before starvation under red-light, even though is highly uncommon. As we expect a similar number of sporophytes as eggs between successive counting days, if the fertilization of eggs was optimal, it is likely that eggs were not properly fertilized under the nutrient conditions utilized. A decrease in gametophytes with eggs was visible for the majority of the Bodø population, from t5 to t20.

There seem to have been a mismatch in time for when females became fertile, which could lead to eggs deteriorating without becoming fertilized. A similar response has been reported previously (Martins *et al.* 2017) with a population from Helgoland, where there initially was an unbalanced sex-ratio with surplus of females and with males possibly not producing enough sperm to fertilize all eggs. This is similar to the Bodø population where there also were less males (approx. 30%) than females suggesting that there simply were not enough fertile males releasing spermatozoids. Alternatively, the unsuccessful formation of juvenile sporophytes might be explained by a mismatch in time of fertility, as sperm generally only survive for around 12 hours after release (Li *et al.* 2013). Males are protandric, i.e., they become fertile before the females and wait for the females to release their eggs and pheromone (Martins *et al.* 2017; Silva *et al.* 2021), which is likely to have affected the final sporophyte recruitment at t21 for both populations.

As nutrient availability (N and P) decreased throughout the experiment, the females possibly did not continuously produce new oogonia and eggs. Additionally, during the counting procedure, eggs were not considered to be present if they lacked colorization and were not attached to the female oogonia, which could potentially present some experimental bias as eggs may yet be present but out of focus. Contrary to Bodø, the Quiberon experiment, had more males (approx. 20%) than females which could explain the higher total percentage (7%) of sporophyte development, as this surplus may have facilitated fertilization of released eggs (Martins et al. 2017). Furthermore, the use of AS with addition of nitrate and phosphate have the possibility of altering the ontogenetic development achieved for this study. Although similar studies (Silva *et al.* 2021) used similar contents (10 µM N and 1 µM P), it is yet considered low compared to the other studies (Lüning, 1980; tom Dieck, 1992).

4.2 Recruitment of juvenile sporophytes

For both populations, temperature is the leading factor in altering the recruitment of juvenile sporophytes as it was highest at 8 and 12°C for both populations. This was irrespective of the nutrient concentrations for the Quiberon population only. For Bodø, high nutrients had a significant effect on the sporophyte formation as well, with temperature and nutrients both affecting the recruitment. However, the factors did not have any interactive effects, but rather single effects on the final sporophyte recruitment. Indicating that both temperature and nutrients had an effect on the final sporophyte recruitment for the Bodø population at t21.

Considering Quiberon, within the optimal temperature range (8 and 12°C), the highest percentage of sporophytes recruited was 7% in high nutrient (HN) treatment at 12°C, while few

to no sporophytes were observed at suboptimal temperatures (4 and 8ºC) at LN treatment. Recruitment observed was extremely low and much less than observed in other study dealing with *L. digitata* and other kelps (Gauci, 2020; Martins *et al.* 2017).

Some kelp species have the ability to delay development of spores or gametophytes for many months when essential nutrients, for instance iron and nitrate, are missing (Carney and Edwards, 2010), however upon periods of sufficient quantities of nutrients sporophyte production can be accelerated in the matter of a few days (Carney *et al.* 2013). This might also explain part of the current results, if nutrients were resupplied to the replicate's halfway through the experiment, the sporophyte recruitment could be higher for replicates with the HN treatments. Thus, the gametophytes could have been under a delayed or inhibited state of development under poor environmental conditions through the experiment conducted (Carney and Edwards, 2010; Carney *et al.* 2013). Similar studies (Silva *et al.* 2021) shows that *L. digitata* gametophytes became reproductive upon recovering in spring conditions, after having delayed development under unfavorable conditions. This differs from other kelp species, such as *Alaria esculenta*, in which a generation of *L. digitata* is not only limited to a single year (Silva *et al.* 2021).

Towards the end of the experiment, higher temperatures (18ºC) proved to be sub-lethal and led to deterioration of gametophytes, thus stopping the process of gametogenesis. As observed by tom Dieck (1992), where temperatures ranging from 18-21°C resulted in gametogenesis becoming suppressed and no juvenile sporophytes were formed. Our sporophyte recruitment at these temperatures match with previous studies (Martins et al. 2017) with juvenile sporophyte recruitment at <1% at 18ºC. Leading to decreased survival of gametophytes at higher temperatures with low ontogenetic development. The different responses and interactions of each population to the nutrient and temperature treatments are presumably explained by local adaptation, as it may be each population exhibiting functional differences based on the nutrient availability and temperature gradients of each their native environments. In terms of cells per female gametophyte, the females of Quiberon had more cells (6.1± 0.66) than the Bodø females (5 ± 0.4). Even though there was significant difference, it could still influence the number of those cells becoming fertile, as each female cell has the equal possibility of producing sporophytes.

The expectation was that Bodø would have a lower temperature optimum for recruitment than Quiberon as Bodø never experiences temperatures above 12°C (4 – 12°C) while Quiberon thrives in 9 – 18°C waters. This was observed through the experiment, where most sporophytes in Bodø developed at 4 and 8°C HN and 4°C LN while most sporophytes in Quiberon are at 8 and 12°C HN and 12°C LN. The more optimal temperatures also allowed good sporophyte production under LN. Although these data could not be evaluated statistically there is an obvious pattern. It shows that both populations would likely rather reproduce in winter to spring than in the summer, this is especially evident for Quiberon. Although both populations seem be slightly locally adapted, they both inherit the same response range. Liesner *et al.* (2020) noted that L. digitata populations at the southern-most distributional gradient, such as Quiberon, present slight physiological advantages to higher temperatures than more northern populations. However, they may yet react negatively with the prolonged summer temperatures (21-23°C) projected by the end of century (Müller *et al.* 2009; Oliver *et al.* 2018) in terms of both gametophyte growth and sporophyte reproduction (Bartsch *et al*. 2013; Lüning, 1980). Which according to our result, could be detrimental for the coming generations as sporophyte formation at sub-lethal temperatures such as 18°C was minimal.

4.3 Gametophyte growth

For gametophyte vegetative growth, sufficient quantities of macronutrients such as nitrogen, phosphate and iron are crucial for many kelp species (Carney and Edwards, 2010; Roleda, 2015). Gametophyte growth was observed across all treatments, with higher growth in terms of gametophyte size for all gametophytes in high nutrient treatments. Interestingly, gametophytes at suboptimal temperatures also grew well and growth was even highest at 18°C. The latter might have been reinforced by two processes: inhibited gametogenesis and partial deterioration of gametophytes which opened up space for competitors and also may have generated additional nutrients for growth. Gametophytes at a later stage in the experiment were starting to deteriorate at higher temperatures (18°C) even though some were looking large and healthy. One effect of this is shown with the larger gametophytes being present in BOD-HN-18 towards the end of the experiment However, gametophytes in LN at 18°C, did not see as large of an increase in size. Martins *et al.* (2017) recorded that vegetative gametophyte growth was ideal at between 10-18°C which is similar to the range of this study, where the largest gametophytes were measured between 12-18°C.

4.4 Nutrient treatments

Nutrient treatments were based on local data of annual concentrations (Ibrahim *et al.* 2014; Wassmann *et al*. 2000), and the high nutrient treatment (15 µmol L-1 N: 0.5 µmol L-1 P) resulted in a 30N:1P ratio which is considered to represent the optimal ratio for growth of seaweeds including kelp (Harrison and Hurd, 2001). However, even though nutrient concentrations were based on local data from each of the sites (QUI/BOD), the high nutrient treatment used in this study is still much lower than most other studies dealing with gametogenesis in kelps and has been considered 'low nutrient concentration` in Gauci (2020), Liesner *et al.* (2020), Martins *et al.* (2017), Schimpf (2021).These authors used Provasoli enriched seawater (PES; Provasoli, 1968), half strength PES, or natural concentrations of normal seawater which in Martins *et al.* (2017) was at ~18 µmol L-1 NO_{3-} for low nutrient treatments. Thus, the concentrations we used for HN and LN could influence the ontogenetic developmental response of the gametophytes in the study. F2 and PES (Provasoli, 1968), both contain nitrate concentrations between 500-600 µmol L-1 (Martins *et al.* 2017), which makes the concentrations used in our study look considerable low. However, other studies have used lower concentrations to study growth and development (Boderskov *et al* 2016; Forbord *et al.* 2021; Handå *et al*. 2013), with nitrate concentrations varying between 2-18 µmol L^{-1} based on local data for kelp species. Making our high concentration (15 µmol L^{-1} N), a justified concentration to use but could also present a factor of differential growth and ontogenetic development when compared to studies using other nutrient enrichment mediums. The nutrient concentrations at the end of the experiment show high variance in PO_4^{3-} values markedly above the initial, which likely is due to gametophytes deteriorating towards the end of the experiment as was observed during counting by discoloration and disintegration of gametophytes. As a consequence, nutrients were likely released into the medium, which resulted in PO_4^{3-} concentrations for the BOD-HN-18 replicates being higher than the initial value (0.5 µmol L-1). The overall effect of the nutrient treatments on the ontogenetic development of the gametophytes was low, which may be an effect of not renewing the medium after experimental start. Nevertheless, for HN treatments, only nitrates were utilized by the gametophytes for growth and ontogenetic development.

4.5 Local adaptation

Furthermore, results showed that the populations considered for this study; Bodø and Quiberon, are affected differently when exposed to the same nutrient and temperature treatments. Material from each population was kept under the same long-term conditions in the laboratory before

experimental start, under red-light and half-strength PES (Provasoli, 1968). Thus, local adaption is considered the explanation as responses are related to the genetics from each distinctive site (QUI/BOD) and not laboratory acclimation. Schimpf (2021), discovered that survival of *L. digitata* gametophytes from the southern-most population, Quiberon and northern-most population, Spitsbergen, responded best to their *in-situ* annual SST of each respective site. Which suggested that have each of the populations showed partial local adaptation according to each site's local environmental conditions considering gametophyte survival (Schimpf, 2021).

The surrounding environment of each of the sites have different fluxes and influences of both temperature and nutrients, which could reflect functional differences between the two populations (Aure and Skjoldal, 2004; Ibrahim *et al.* 2014; OSPAR, 2018; Wassmann *et al*. 2000). According to OSPAR (2018), nutrient inputs in the Bay of Biscay are lower, about half, of inputs in the greater North Sea. Additionally, are riverine inputs into the sea strongly affected by regional flood events which are coupled with a downward trend in phosphorous (10-12 kilotons/year) inputs since 1997. The flood events make the nutrient composition near the Bay of Biscay quite variable and have experienced lower nitrate concentrations since 2000 (~137-300 kilotons/year). Kelps are therefore likely affected which could be an effect of the Quiberon population relying less on nutrients due to its proximity to the Bay of Biscay and the observed downward trend in phosphorus. Which may be why Quiberon is not having a significant response of the nutrient treatments on its recruitment (OSPAR, 2018).

Considering the Bodø population, which had a significant response to the nutrient and temperature treatments with respect to recruitment (Table 5), the trends in local nutrient inputs are considerably different from that of Quiberon (Aure and Skjoldal, 2004). The Norwegian coastal current (NCC) flows alongside the northward coast and deposit nutrients with no specific hot spots, bringing concentrations of phosphorus and nitrogen. Although these concentrations are low enough not to be considered with eutrophication, they nevertheless have increased due to anthropogenic activity such as aquaculture, bringing additional nutrients to the regions (Aure and Skjoldal, 2004). Even though annual concentrations are lower at Bodø than at Quiberon (Aure and Skjoldal, 2004; OSPAR, 2018) , the recent increase in nutrients as a result of fisheries etc. have deposited more nutrients in the northern part of Norway than what accumulates naturally. This could then present each population with functional differences, with one site experiencing a downward trend in nutrients and another experiencing an upward trend (Aure and Skjoldal, 2004; OSPAR, 2018).

The present circumstances make it clear that more studies on nutrient concentrations and temperature regimes in relation to species development needs to be considered for further research. In reference to how the different factors affect one another and ultimately the effect they have upon a certain species. This is in regard to future climate scenarios and if a species is able acclimate, survive, and reproduce.

In conclusion our results define the importance of increasing the knowledge of *Laminaria* species response to different temperature and nutrient regimes, with emphasis on the effects from the environment on gametophyte growth, recruitment, and survival. Populations exhibit different responses according to their local histories in terms of the effects of their respecting optimum temperature and nutrients treatments on sporophyte formation. Although both populations seem be slightly locally adapted, they both inherit the same general response range for gametogenesis. This is an indication that certain changes to the environmental factors examined (temperature and nutrients) can have large effects on an array of different developmental phases of the kelp, both within and between populations. The survival of these complex canopy-forming bioengineers is depending on a common understanding on how climate change affects them and their life cycles. With more nutrients entering the water through anthropogenic exploits, natural balances of resources are likely to be altered. Further studies on the response of different nutrients and how it influences different aspects of the life cycle of kelps are in dire need, including a broader geographical range under different nutrient and temperature treatments.

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Supplementary data

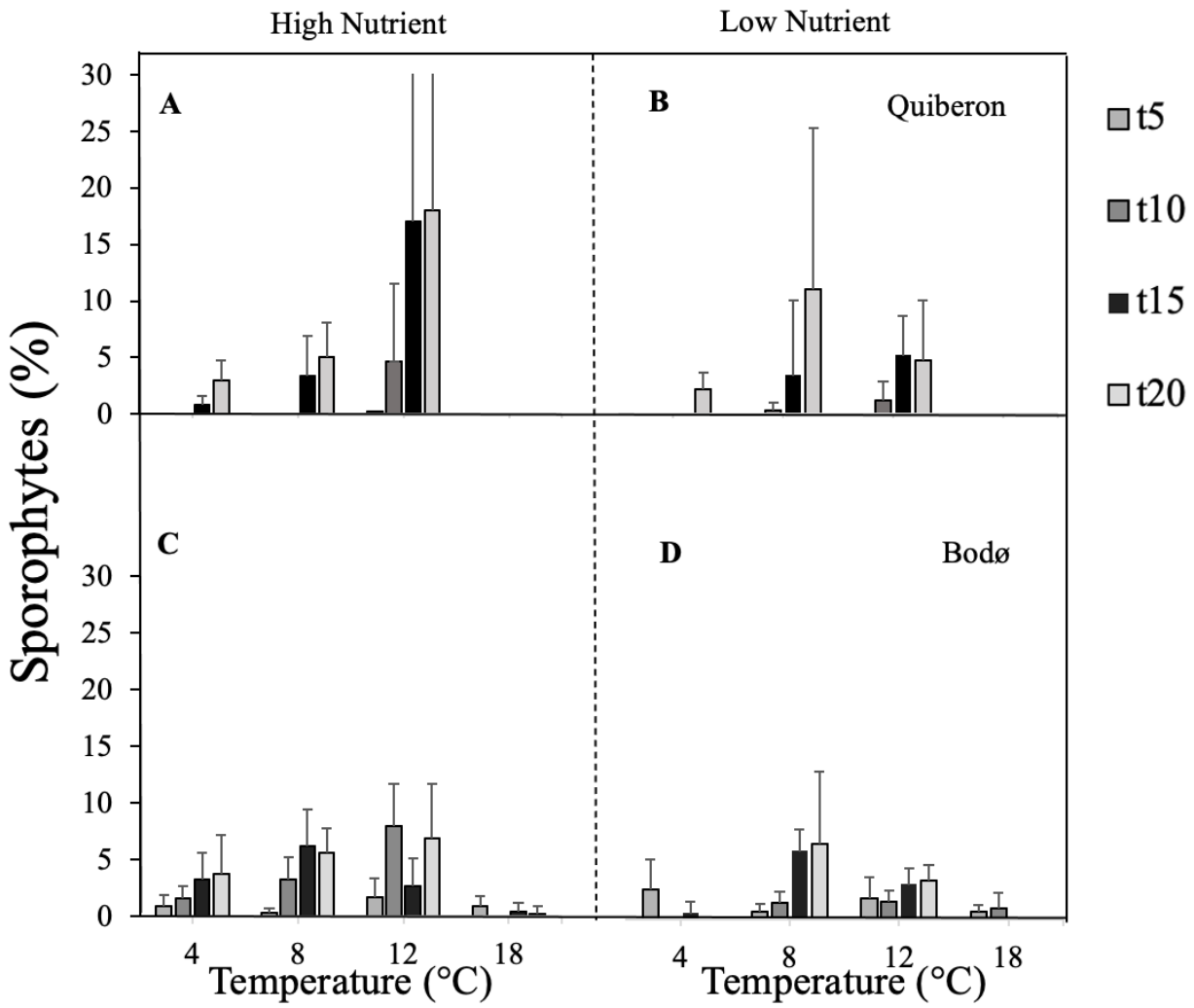

Figure S2: Percentage of female gametophytes with juvenile sporophytes, under high and low nutrient and temperature treatment over 20 days (BOD vs QUI) (mean ± standard deviation, n=5).

Population	Parameter	SST (°C)				PO4		NO3		N:P ratio	
		Min	Max	Avg Winter	Avg Summer	Min	Max	Min	Max	Min	Max
Bodø, NO	**Measure**	3°C	15°C	4°C	12°C	0.2-0.3µM	0.6 µM	0-2 µM	12µM	7	20
(67.28°N)	**Month**	March	July	March	August	April-June	March	June-August	Jan-March	June	Jan-March
	Year	2011-2021		2011-2021		March-October, 1996		1997-2010		1996-2010	
	Type of Value	Monthly mean		Monthly mean		Every 4 days		Post bloom, Gimsøy	Winter, Gimsøy	daily	
		(past 10 years)		(past 10 years)		Tromsø		Middle sample part	Middle sample part		
	Reference	htttp://www.seatemperature.info				Wassmann *et al.* 2000		Ibrahim *et al.* 2014			
Fornæs, DK	Measure	-1.2°C	22.8°C	2.6°C	18.3°C	0.01 µM	0.57 µM	0.17 µM	7.34µM	17	13
(56.26°N)	Month	February	July	February	August		late autumn-winter		late autumn-winter		Late autumn/winter
	Year	2011-2021		2011-2021							
	Type of Value	Monthly mean (10yr, Grenaa)		Monthly mean (10yr, Grenaa)		Low		Low	Naturally high winter conc.		
	Reference	htttp://www.seatemperature.info				(Boderskov *et al.* 2015)			(Boderskov *et al.* 2015)		
Helgoland, GE	Measure	4.1°C	17.59°C	4.5°C	18.4°C	0.21µM	0.72µM	1.43µM	18.4µM	7	26
(54.10°N)	Month	Feb	Aug	Feb	Aug	May	Feb	August	Feb	Aug-Oct	April-May
	Year	2010-2018		2011-2021		2010-2018		2010-2018		2010-2018	
	Type of Value	Monthly mean (daily values)		Monthly mean (10 years)		Montly mean (daily values)		Monthly mean (daily values)		Monthly mean (daily values)	
	Reference	(K. Wiltshire)		htttp://www.seatemperature.info		(K. Wiltshire)		(K. Wiltshire)		(K. Wiltshire)	
Quiberon, FR	**Measure**	7.1°C	20.3°C	9.2°C	18.1°C	0.05µM	0.54µM	0.14µM	28µM	3	52
(47.29°N)	**Month**	Feb	July	Feb	Aug	June	Feb	July	Feb	June-July	Feb
	Year	2011-2021		2011-2021		2019	2020	2019	2020		
	Type of Value	Monthly mean (10 years)		Monthly mean (10 years)		Monthly mean (3 values/month)		Raw measurement data	Raw measurement data		
	Reference	htttp://www.seatemperature.info				http://ww.somlit.fr/mysomlit/		http://ww.somlit.fr/mysomlit/			

Figure S3: Experimental set-up with nutrient and temperature conditions from the study sites Bodø and Quiberon. Fornæs, Denmark and Helgoland, Germany shown for comparison and as intermediate conditions between the study sites. Table shows SST (min, max, average winter, and average summer), PO_4^{3-} (phosphate; minimum and maximum), NO_{3-} (nitrate; minimum and maximum), and N/P ratio (minimum and maximum). Data used in experimental design and set-up