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Interactions of daylength, temperature and nutrients affect thresholds for life stage transitions in the kelp *Laminaria digitata* (Phaeophyceae)

DOI 10.1515/bot-2016-0094

Received 21 August, 2016; accepted 28 February, 2017; online first 31 March, 2017

Abstract: Kelp beds worldwide are under pressure from ongoing climate and environmental change. Along European coastlines increases in seawater temperature and changes in nutrient conditions occur where upwelling events are disrupted and also along eutrophicated coasts. In addition, seaweed responses to change may interact with seasonal daylength cycles. We performed a factorial experiment to examine the combined effects of seawater temperatures, nutrient regimes and photoperiod (long and short days) in order to better understand how latitudinal or seasonal differences in daylengths affect the sensitivity of transient microscopic kelp stages of *Laminaria digitata* from the North Sea to warming and eutrophication. While the optimal temperature range for vegetative gametophyte growth was 10°C–18°C under long summer photoperiod conditions, gametogenesis was induced at lower temperatures between 5°C and 15°C, with maximum sporophyte development under long photoperiods and enriched nutrient regimes, which represent local late spring conditions. Although gametogenesis was fastest at 10°C–15°C, sporophyte recruitment was highest at 5°C. As these particular early life cycle processes in *L. digitata* have different temperature optima, this may drive the seasonal cycle of recruitment in the field. Increasing summer temperatures due to global warming

will increase gametophyte size due to enhanced vegetative growth and inhibition of gametogenesis. This will probably lead to delayed but enhanced recruitment of new sporophytes under cooler autumn to spring conditions over a wide geographical scale, preventing the formation of juvenile sporophytes under stressful summer conditions and possibly changing annual recruitment patterns.

Keywords: gametogenesis; nutrients; photoperiod; recruitment; temperature.

Introduction

The order Laminariales houses the bulk of the macroalgal species known as kelp, which play critical habitat-structuring roles, protecting coastlines against erosion and providing food, shelter and nurseries for a diverse array of marine organisms, supporting a wealth of biodiversity and ecosystem services (Steneck et al. 2002). Kelps are also economically important biota, providing many food products, hydrocolloid compounds, bioremediation agents, as well as potential biofuel feedstock (Bartsch et al. 2008, Mouritsen 2013). The ecological and socio-economic value of ecosystem services provided by these species is poorly estimated, although kelp forests in Australia were estimated to account for more than 15% of regional economies (Bennett et al. 2016). In Europe, kelp cultivation and research are increasing fields of interest (Sanderson et al. 2012, Kerrison et al. 2015), although management strategies able to cope with climate changes are sorely needed.

The Laminariales exhibit an obligate heteromorphic life cycle that alternates between microscopic stages (spores, gametophytes, gametes and microscopic sporophytes) and macroscopic sporophytes (reviewed in Lüning 1990, Schiel and Foster 2006). Mature diploid sporophytes release haploid spores that settle and develop into dioecious (i.e. separate female and male) gametophytes, which undergo gametogenesis under favourable conditions

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(Bartsch et al. 2008). Fertilisation ensures recruitment via the development of a new generation of microscopic diploid sporophytes. Microscopic stages determine recruitment success but are highly susceptible to various environmental perturbations (e.g. Wiencke et al. 2006, Fredersdorf et al. 2009) and thus are considered the most critical stages impacting species distributional limits (reviewed in Schiel and Foster 2006, Oppliger et al. 2012). Microscopic kelp stages are exposed to different environmental conditions than their macroscopic sporophytes as they are probably growing in the very shaded sub-canopy of parental plants or endo-/epiphytically (Garbary et al. 1999, Lane and Saunders 2005). Thus, each life cycle stage requires specific environmental conditions for growth and development (e.g. Floc'h et al. 1991, Gómez and Wiencke 1996). However, most studies of environmental drivers for growth and reproduction focus on a single life stage, and therefore new approaches are needed to integrate the environmental effects across life histories (Russell et al. 2012).

Under non-lethal environmental conditions unfavourable for gametogenesis, gametophytes may remain vegetative and viable for several months, delaying gametogenesis until conditions are conducive (tom Dieck 1993, Carney and Edwards 2010). Thus, gametophytes have been suggested to play an important role analogous to terrestrial plant seed banks (Santelices 1990, tom Dieck 1993), and may be particularly important for kelp populations that experience sporophyte die-off due to seasonal perturbations or large-scale disturbances (Ladah and Zertruche-González 2007, Barradas et al. 2011).

Strong seasonal variability in environmental conditions characterises temperate regions: while in summer the nutrient content of the seawater is often low and temperature, irradiance and daylength (photoperiod) are high, in winter these parameters are reversed (Lüning 1993). Pronounced changes in daylength between seasons are more characteristic of the Northern Hemisphere, which contains more coastlines at high latitudes, but daylength changes are also generally relevant for comparison of kelp forests stretching along latitudes independent of season. Exceptions occur in areas with summer upwelling such as Iberia and north west Africa, allowing persistence of isolated cold-temperate seaweed canopies (e.g. Assis et al. 2016, Lourenço et al. 2016) as summer temperatures are colder and nutrient supplies are higher than in areas without upwelling. Recent reduced upwelling has been implicated in the decline of European kelp beds off the northern coast of Spain (Fernández 2011, Voerman et al. 2013).

Most *Laminaria* species are so-called season anticipators, as they grow and reproduce with an annual rhythm controlled by specific seasonally recurring environmental

triggers (Kain 1989). Therefore, climate-driven changes in temperature and/or nutrients in combination with seasonal photoperiods may alter the response to these environmental triggers (Lüning 1980b, Lüning and tom Dieck 1989) potentially interfering with, or disrupting life history cycles. Empirical data suggest that growth and reproduction of gametophytes are greatly affected by nutrient availability (e.g. Hsiao and Druehl 1973, Hoffmann et al. 1984), which fluctuates seasonally. Data also suggest that kelp gametophytes arrest their development under low nutrient conditions and re-establish it when conditions improve (e.g. Carney and Edwards 2010, Morelissen et al. 2013). The seasonal variation of daylength plays an important role in controlling development and reproduction in macroalgae, as light-dark cycles are responsible for triggering the onset of the different life cycle phases (Lüning 1980b, Lüning and tom Dieck 1989) of many species. In kelps, this was demonstrated for *Undaria pinnatifida*, which requires long days (16:8 h light:dark) for gametophyte growth, and short days (8:16 h) for gametogenesis (Choi et al. 2005). In some kelp species, gametogenesis occurs only under extreme short days (<8 h light) and low temperatures (tom Dieck 1989) and the induction of sporophyte fertility is also under photoperiodic control in some species (Lüning 1988, tom Dieck 1991).

Most studies on early developmental stages have examined the effects of a single environmental factor, however in the field kelps are subjected to a multitude of factors, which can change on seasonal, temporal or spatial scales. Studies evaluating interactive synergistic or antagonistic effects of multiple environmental factors on development of microscopic life stages of kelps have recently increased (Fredersdorf et al. 2009, Zacher et al. 2016), but interaction with seasonal photoperiods have scarcely been considered (Mohring et al. 2013, Mansilla et al. 2014). In *Ecklonia radiata* seasonal changes of temperature and daylength affected early survival and growth of sporophytes (Mohring et al. 2013). In *Lessonia flavicans* a combination of summer photoperiods (18:6 h) and temperatures (9°C) maximised sporophyte biomass gain (Mansilla et al. 2014), while biomass increase was minimal in sporophytes cultured in winter photoperiods (6:18 h) and temperatures (5°C).

Laminaria digitata (Hudson) J.V. Lamouroux is a perennial North Atlantic species in the order Laminariales, growing in the lower intertidal and sublittoral fringe of rocky habitats. In the north east Atlantic its distribution ranges from the Arctic (Svalbard) to cold-temperate southern Brittany, France (Lüning 1990). The sporophyte phase of *L. digitata* grows best at 10°C–15°C (tom Dieck 1992) but reproduces optimally at 5°C–10°C (Bartsch et al. 2013). In contrast, the gametophyte phase grows best at 15°C–18°C (Lüning 1980a), although gametogenesis is optimal at 11°C

(tom Dieck 1992). Reproduction thus has a lower temperature window than growth in both life cycle phases and sporophytes need lower temperatures than gametophytes for vegetative growth. These processes were only studied from one locality (Helgoland, North Sea), but the general relation may be similar across populations throughout the distribution range, although differentiated populations could have evolved distinct responses.

Basic knowledge concerning temperature regulation already suggests that seasonal regulation of life cycle processes differs between gametophytes and sporophytes, and probably drives annual patterns of population recruitment. The impact of simulated current and increasing temperature scenarios, combined with changing nutrient conditions and photoperiods expected to co-occur over the course of the year have not been investigated in any given kelp species. Here we address this question, using the North Atlantic keystone kelp species *L. digitata* as model organism. We focused on the performance of microscopic life cycle stages and the resulting recruitment capacity of this species, allowing for assumptions of changing annual recruitment patterns under global change scenarios. This study will provide valuable baseline information to better understand the impacts of seasonality and co-occurring environmental changes on recruitment capacity of kelps, to help predict possible shifts in recruitment and persistence under changing environmental conditions, and to aid conservation strategies and cultivation practices of an ecologically and economically valuable kelp species.

Materials and methods

Algal material

Fertile sporophytes of *Laminaria digitata* can be generally found in the field between April and December with a maximum in late summer, but some populations are fertile year-round (Sjötun and Schoschina 2002, Bartsch et al. 2008, 2013). Eight sporophytes of *L. digitata* were collected during low tide from Helgoland (North Sea, Germany) in September 2015 and transported to Bremerhaven on ice. Sori were cleaned and zoospores from each individual were released separately into sterile seawater, before being transferred to Petri dishes and maintained at 15°C under 3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of red light (LED Mitras daylight 150 controlled by ProfiLux 3, GHL Advanced Technology, Kaiserslautern, Germany), 16:8 h light:dark cycle in 10 ml l^{-1} Provasoli enriched seawater (PES; Provasoli 1968) for 1 month and then in 20 ml l^{-1} PES until the start of the

experiment. The seawater was changed monthly. Under these conditions small vegetative gametophytes developed.

Experimental setup

A factorial laboratory experiment was performed combining the three factors daylength (16:8 h light:dark = LD and 8:16 h light:dark = SD), nutrients (North Sea seawater with and without addition of 10 ml l^{-1} PES, for nutrient enriched [=HN] and nutrient poor conditions [=LN], respectively) and temperature (5°C, 10°C, 15°C and 18°C \pm 0.5°C). Irradiance was set to 12–15 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (white LED light) measured with a LI-COR LI-185B Photometer (LI-COR-Biosciences, Lincoln, NE, USA). Temperature and photoperiod conditions were selected to reflect environmental conditions for winter (5°C and 8:16 h light:dark) and summer (15°C and 16:8 h) expected in a wide distribution range of the species. Seawater temperatures of 10 and 18°C were used to reflect general seasonal (spring and autumn) and/or global warming scenarios during winter and summer conditions, respectively. As the experiment stretched over several weeks, we chose a sub-lethal summer scenario instead of 20°C, which might be lethal for juvenile sporophytes (tom Dieck 1992). The nutrient content was not specifically determined. The seawater used was winter water from the southern North Sea which generally is quite nutrient rich (Skjoldal 1993) while our high nutrient treatment was supplemented with 10 ml l^{-1} PES and therefore was super-saturated with nutrients (>137 $\mu\text{mol NO}_3^- \text{ l}^{-1}$; Sarker et al. 2013). Thus the low nutrient conditions possibly did not simulate depleted conditions but the high nutrient condition simulated eutrophic sites (Moy and Christie 2012, Norderhaug et al. 2015).

Vegetative female and male gametophytes derived from all eight *L. digitata* individuals were scraped from the Petri dishes, combined and diluted with sterile seawater to prepare a gametophyte stock solution. Stock solution of 2 ml were added to small plastic Petri dishes ($\varnothing=5.3$ cm) containing 12 ml of sterile nutrient-poor or nutrient-enriched seawater in order to obtain a gametophyte density of ~ 150 gametophytes cm^{-2} . Four replicate Petri dishes were used for each treatment (16 treatments \times 4 replicates = 64 Petri dishes in total). Eight additional Petri dishes were prepared: four replicates were used to measure the gametophyte area on day 0, so that the treatment replicates did not experience initial stress, and the remaining four replicates were maintained at 15°C in darkness for 20 days. Culture medium was changed on day 10 by the replacement of 5 ml of nutrient-poor or enriched seawater to each Petri dish. The total duration of the experiment was 20 days.

Gametophyte growth, ontogenetic stages of gametogenesis and sporophyte recruitment

Gametophyte growth

Gametophyte area was quantified on day 0 and day 7 by processing photographic data obtained from an Olympus CKX41 inverted microscope (Olympus Co., Tokyo, Japan) with a Canon EOS 550D digital camera (Canon, Tokyo, Japan), using ImageJ software (Schneider et al. 2012). Thirty fields of view per replicate were randomly photographed, and the area of all female and male gametophytes present in each field of view (3.94 mm^2) was determined. All areas per replicate were summed and standardised (mm^2 gametophyte cm^{-2}). If a female gametophyte became fertile and formed oogonia, the area of the oogonia was included. On day 0 only four replicates were measured assuming that the starting point for all treatments was the same. The gametophyte area data were evaluated by 3-factor ANOVA (fixed factors: daylength, nutrients, temperature) using SPSS statistical package (v. 23.0, SPSS Inc., Chicago, IL, USA). When significant interactions between treatments were observed, *post hoc* comparisons were performed (Tukey's multiple range test) to determine the responsible factor levels. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) before performing the statistical analysis.

Quantification of ontogenetic stages

The percentage occurrence of four ontogenetic stages of female gametophytes (vegetative, oogonia, egg release and sporophyte formation) was quantified every 5 days over a period of 20 days in ≥ 300 female gametophytes per replicate using an inverted microscope. For each female gametophyte the latest developmental stage was recorded. Gametophytes were considered to be in the oogonia, egg release or sporophyte stage if at least one cell per gametophyte had entered this developmental stage. Juvenile sporophytes were differentiated from released eggs if a first cell division was visible. Microscopic sporophytes were discriminated into two categories: "1" Sporophytes with normal morphology (regular cell divisions, clear polar differentiation into basal rhizoid and proximal elongated blade), which normally stay attached to the respective female oogonium, were considered to represent fertilised diploid sporophytes. "2" Unattached sporophytes with non-polar irregular morphology, often

with missing rhizoids were considered to represent unfertilised partheno-sporophytes that would not fully develop into healthy sporophytes and thus represent unsuccessful recruits. These classes were defined *sensu* tom Dieck (1992, figures 9–14) who fertilised female and mixed gametophytes in separated cultures of diverse *Laminaria* species and always observed partheno-sporophytes when eggs developed without males present and normal sporophytes when both sexes were present.

Recruitment of juvenile sporophytes

Recruitment capacity of juvenile sporophytes was quantified after 20 days of culture with three distinct measures: (1) percentage of female gametophyte fragments with sporophytes, (2) absolute number of normal type "1" sporophytes per cm^2 , and (3) absolute number of unattached and/or irregular type "2" sporophytes per cm^2 considered to represent partheno-sporophytes *sensu* tom Dieck (1992). The absolute number of all sporophytes, i.e. normal type "1" sporophytes, and type "2" partheno-sporophytes was also quantified. This was considered as a separate parameter for the potential overall gametophyte fertility that has taken place. The total number of type "1" and "2" sporophytes was evaluated using a stereo microscope Olympus SZX10 (Olympus Co., Tokyo, Japan). A total of 15 fields of view ($63\times$ magnification) was analysed per replicate.

Percentage sporophyte data (arcsin square root transformed) and sporophyte density data (logarithmically transformed) were evaluated by 3-factor ANOVA (fixed factors: daylength, nutrients, temperature) followed by Tukey's multiple range *post hoc* test to determine which factor level was responsible for specific treatment differences (SPSS, v. 23.0, SPSS Inc., Chicago, IL, USA). As sporophyte formation was near zero at 18°C , this treatment was excluded from the analyses as data did not meet assumptions of normality and homogeneity of variance.

Results

Gametophyte growth

Results of the experiment examining the interactive effects of temperature, nutrients and daylength on gametophyte growth showed that the initial surface area of female and male gametophytes together was $0.44 \pm 0.05 \text{ mm}^2 \text{ cm}^{-2}$ (mean \pm SE), and did not differ significantly after

1 week in the dark control (15°C and low nutrient treatment; $0.46 \pm 0.03 \text{ mm}^2 \text{ cm}^{-2}$; t-test, $p = 0.658$). After 7 days, the surface area of female and male gametophytes had increased in all treatments, ranging from 0.58 to $0.95 \text{ mm}^2 \text{ cm}^{-2}$ (Figure 1) and reaching a maximum at 15°C under long day (16:8 h light:dark cycle, LD) and high nutrients (HN). The surface area of gametophytes differed significantly with temperature and daylength, but there were no effects of nutrients or interactions between factors (Table 1). Gametophyte area was significantly greater (~20%) at 10°C–18°C compared to 5°C and was significantly higher (~24%) in all LD compared to SD conditions.

Temporal development of ontogenetic stages during gametogenesis

The temporal development of ontogenetic stages during gametogenesis of *Laminaria digitata* in different

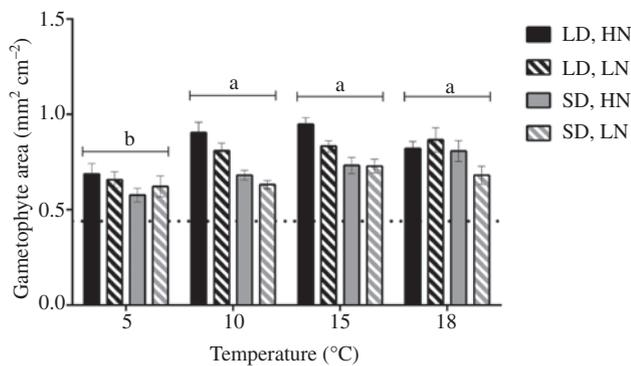


Figure 1: Effects of temperature, nutrient availability and daylength on gametophyte surface area of *Laminaria digitata* after 7 days. Values are expressed as the mean \pm SE ($n = 4$). Dotted line represents the mean value of gametophyte surface area on day 0. Different letters indicate significant differences among the means for each temperature. See Table 1 for results of statistical analysis.

Table 1: Three-way ANOVA for the effects of temperature, nutrients and daylength on the surface area of *Laminaria digitata* gametophytes after 7 days.

Factor	df	SS	MS	F	p-Value
Temperature	3	0.299	0.100	12.97	<0.001
Nutrients	1	0.027	0.027	3.53	0.066
Daylength	1	0.284	0.284	36.97	<0.001
Temperature \times nutrients	3	0.015	0.015	0.63	0.600
Temperature \times daylength	3	0.040	0.040	1.74	0.171
Nutrient \times daylength	1	0.001	0.001	0.12	0.734
Temperature \times nutrients \times daylength	3	0.049	0.049	2.13	0.108

Significant ($p < 0.05$) interactions or main effects are highlighted in bold. df, Degrees of freedom; SS, sum of squares; MS, mean sum of squares; F, F-statistic.

temperatures, daylengths and nutrient conditions is shown in Figure 2. By day 5, a considerable proportion (4%–63%) of female gametophytes had already formed oogonia and eggs at 10°C and 15°C, particularly under LD conditions. The highest proportions of gametophytes with oogonia and eggs were observed at 15°C, LD and HN conditions (32% with oogonia and 31% with eggs). In contrast, under SD and low nutrient (LN) conditions the development of these stages was delayed at all temperatures. At 5°C, female gametophytes became fertile only after 10 days with 26% bearing oogonia and 39% eggs, while at 18°C the proportion with oogonia and eggs did not exceed 23%, regardless of daylength and nutrient treatment. Although female gametophytes at 18°C had developed oogonia in 9%–75% of the gametophytes, by the end of the experiment, they exhibited very few eggs ($\leq 7\%$) in any condition and most of these eggs had not developed further and were disintegrating.

The first microscopic sporophytes were observed on day 10 in all temperatures except 18°C. The highest proportion of sporophytes (43%) developed at 10°C, LD and HN conditions. At 5°C, sporophyte formation in contrast was very low, ranging from 1% to 7%, depending on daylength and nutrient treatments. On the 20th day, almost all female gametophytes cultivated at 5°C, 10°C and 15°C in LD and both nutrient conditions developed a high proportion of sporophytes (87%–100%), while in SD sporophyte formation at these temperatures was mostly lower, ranging between 42% and 89%. Almost no sporophytes ($< 1\%$) developed by the end of the experiment at 18°C, regardless of daylength and nutrient treatments.

Recruitment capacity of juvenile sporophytes

The percentage of female gametophyte fragments with normal juvenile sporophytes (ranging between 42% and

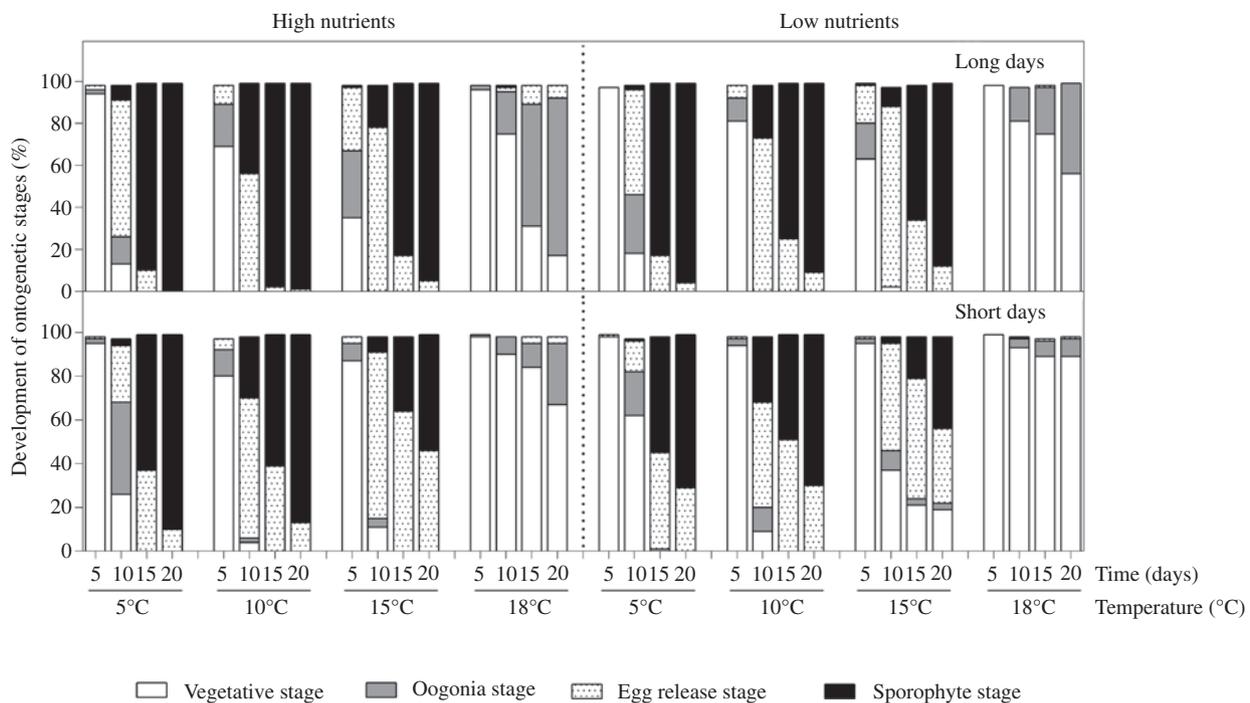


Figure 2: Effects of temperature, nutrient availability and daylength on the development of ontogenetic stages of *Laminaria digitata* over time (mean values, $n=4$).

Counting was done every 5 days over a period of 20 days. SE-values are omitted for clarity.

100%) differed significantly between nutrient conditions and there was a significant temperature \times daylength interaction (Figure 3A, Table 2). At 5°C, 10°C and 15°C in LD, 97%, 94% and 91% of female gametophytes, respectively, had normal sporophytes while the values were significantly lower (~17%–43%) in SD. In general, the proportion of sporophytes was negatively affected by increasing temperatures, decreasing from 97% at 5°C to 91% at 15°C in LD and from 80% at 5°C to 48% at 15°C in SD conditions. In addition, relative sporophyte presence was significantly enhanced by HN (87%) compared to LN (76%).

When absolute numbers of sporophytes with normal morphology and partheno-sporophytes with irregular morphology are considered, recruitment success showed a different and more pronounced pattern (Figure 3B and C, Table 3). After 20 days, the density of normal sporophytes ranged from 620 sporophytes cm^{-2} at 5°C to 415 sporophytes cm^{-2} at 10°C and 221 sporophytes cm^{-2} at 15°C (Figure 3B). Normal sporophyte densities were significantly and ~86% higher in LD than in SD. There was also a significant interaction between temperature and nutrients; a significantly higher number of sporophytes developed under HN compared with LN in all temperatures tested (5°C, 10°C and 15°C). The number of sporophytes in HN was 77% higher than in LN at 5°C, 93% higher at 10°C and 184% higher at 15°C (Figure 3B). Sporophyte recruitment was negatively

influenced by increasing temperature, decreasing significantly from 794 and 447 sporophytes cm^{-2} under HN and LN conditions at 5°C to 327 and 115 sporophytes cm^{-2} under HN and LN at 15°C. In total, recruitment of normal sporophytes was highest at 5°C, LD and HN conditions.

The development of partheno-sporophytes was maximal at 10°C in HN and LD conditions and density ranged from 101 sporophytes cm^{-2} at 5°C to 495 sporophytes cm^{-2} at 10°C and 91 sporophytes cm^{-2} at 15°C (Figure 3C). Partheno-sporophyte densities varied significantly due to the interaction between temperature \times daylength \times nutrients (Table 3). In both daylength and nutrient treatments, the density was significantly higher at 10°C than at 5°C and 15°C. In HN conditions, the formation of partheno-sporophytes was significantly enhanced at 5°C LD, at 10°C LD and SD and also at 15°C SD conditions. On the other hand, the LD regime significantly increased the density at 5°C in HN and at 15°C in both the nutrient treatments.

Total sporophyte numbers (normal sporophytes and partheno-sporophytes) varied significantly due to the interaction of temperature \times daylength \times nutrients (Table 3). The mean density of total sporophytes ranged between 722 sporophytes cm^{-2} at 5°C to 910 sporophytes cm^{-2} at 10°C and 311 sporophytes cm^{-2} at 15°C, with maximal density at 10°C, LD and HN (Figure 3D). Overall, in both daylength and nutrient treatments, the density

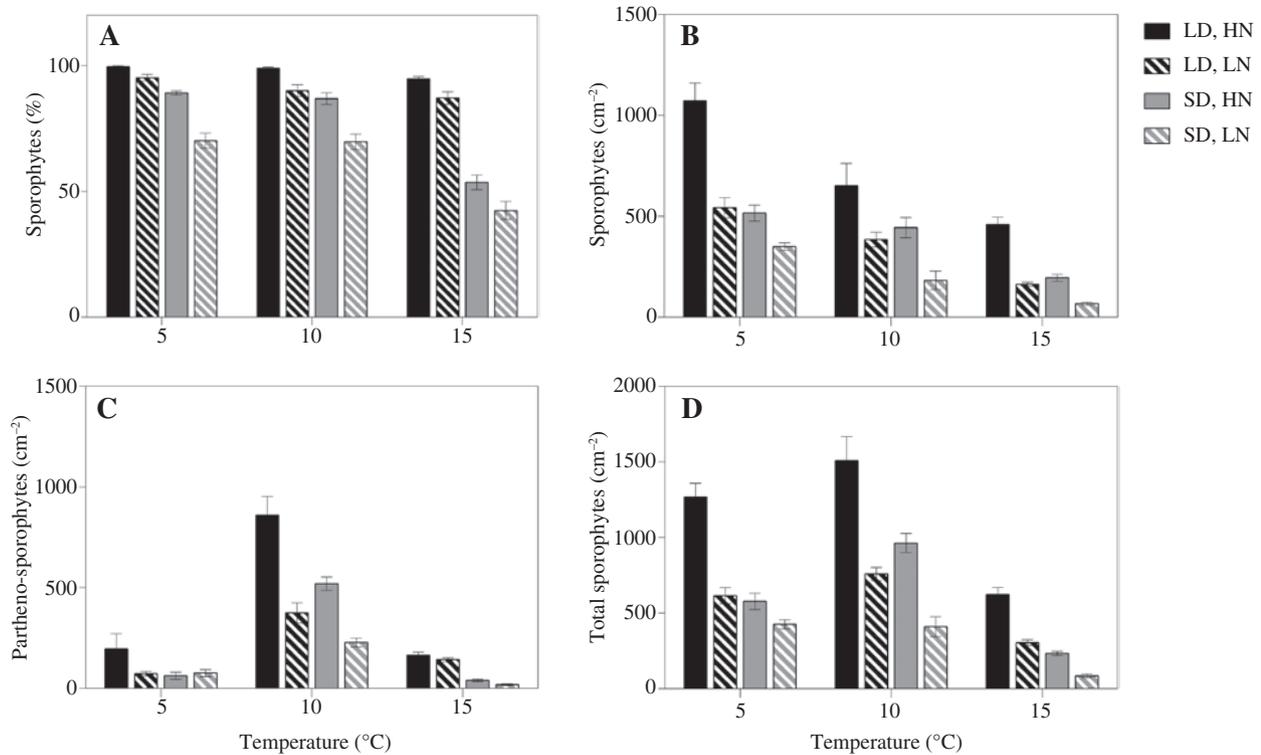


Figure 3: Effects of temperature, nutrient availability and daylength on the recruitment capacity of juvenile sporophytes of *Laminaria digitata* after 20 days.

(A) Percentage of female gametophyte fragments with juvenile sporophytes. (B) Absolute number of normal sporophytes cm⁻². (C) Absolute number of loose lying and/or abnormal sporophytes cm⁻² considered to represent partheno-sporophytes. (D) Absolute number of normal sporophytes and partheno-sporophytes together. Values are expressed as the mean \pm SE (n=4). See Tables 2 and 3 for results of statistical analysis.

Table 2: Three-way ANOVA for the effects of temperature, nutrient level and daylength on the percentage of female gametophyte fragments with juvenile sporophytes of *Laminaria digitata* after 20 days.

Factor	df	SS	MS	F	p-Value
Temperature	2	0.606	0.303	73.940	<0.001
Nutrients	1	0.404	0.404	98.530	<0.001
Daylength	1	1.638	1.638	399.599	<0.001
Temperature \times nutrients	2	0.023	0.011	2.765	0.076
Temperature \times daylength	2	0.124	0.062	15.104	<0.001
Nutrient \times daylength	1	0.000	0.000	0.098	0.756
Temperature \times nutrients \times daylength	2	0.005	0.003	0.657	0.525

Significant ($p < 0.05$) interactions or main effects are highlighted in bold. df, degrees of freedom; SS, sum of squares; MS, mean sum of squares; F, F-statistic.

was significantly higher at 5°C and 10°C than at 15°C. The number of total sporophytes developed under HN was 36%–176% greater than in LN at all temperatures tested (5°C, 10°C and 15°C) and both daylength regimes. Moreover, the total sporophyte density significantly differed between the two daylengths, with approximately 44%–119%, 57%–85% and 167%–259% more sporophytes cm⁻² in LD than in SD under both nutrient treatments at 5°C, 10°C and 15°C, respectively.

Discussion

The current investigation highlights the need to incorporate multiple factors that either co-vary seasonally or along latitudinal gradients as potential drivers of responses to future climate change scenarios. In this study, we show how changing conditions of temperature, nutrients and daylength interact to affect the development of microscopic life stages and influence sporophyte

Table 3: Three-way ANOVA for the effects of temperature, nutrient level and daylength on the absolute number of normal sporophytes per cm², partheno-sporophytes per cm² and total sporophytes per cm² of *Laminaria digitata* after 20 days.

Factor	Sporophytes (cm ⁻²)				Partheno-sporophytes (cm ⁻²)				Total sporophytes (cm ⁻²)						
	df	SS	MS	F	p-Value	df	SS	MS	F	p-Value	df	SS	MS	F	p-Value
Temperature	2	2.123	1.061	90.593	<0.001	2	6.899	3.450	106.666	<0.001	2	2.464	1.232	170.613	<0.001
Nutrients	1	1.354	1.354	115.586	<0.001	1	0.674	0.674	20.834	<0.001	1	1.190	1.190	164.765	<0.001
Daylength	1	1.065	1.065	90.894	<0.001	1	2.031	2.031	62.800	<0.001	1	1.289	1.289	178.523	<0.001
Temperature × nutrients	2	0.104	0.052	4.446	<0.05	2	0.115	0.057	1.777	0.184	2	0.054	0.027	3.756	<0.05
Temperature × daylength	2	0.044	0.022	1.886	0.166	2	0.919	0.459	14.203	<0.001	2	0.172	0.086	11.930	<0.001
Nutrient × daylength	1	0.003	0.003	0.257	0.615	1	0.005	0.005	0.142	0.709	1	0.001	0.001	0.122	0.728
Temperature × nutrients × daylength	2	0.057	0.028	2.420	0.103	2	0.284	0.142	4.385	<0.05	2	0.063	0.031	4.346	<0.05

Significant ($p < 0.05$) interactions or main effects are highlighted in bold. df, degrees of freedom; SS, sum of squares; MS, mean sum of squares; F, F-statistic.

recruitment of an N-Atlantic keystone kelp species, *Laminaria digitata* from Helgoland (North Sea). This allowed us to gain a more integrated knowledge of potential recruitment optima that may vary in space and time and may be used to assist forecasts, management actions or aquacultural purposes.

It has long been known that seawater temperature determines whether kelp gametophytes continue to grow vegetatively or initiate gametogenesis. The combined evaluation of the optimal temperature for both processes has seldom been evaluated; generally the optimal temperature for gametogenesis and gametophyte growth is species-specific and ranges between 5°C and 19°C within the kelp genera *Laminaria* and *Saccharina* (Bartsch et al. 2008). Here, *L. digitata* gametophytes were smaller at 5°C than at 10°C to 18°C, which is in accordance with earlier studies showing that vegetative growth of early *L. digitata* gametophytes was retarded at 5°C, while there was a broad optimum above 10°C, and that growth inhibition occurred near the upper lethal limit of 20°C (Cosson 1973, Lüning 1980a). In the current investigation gametophyte growth of *L. digitata* was also positively related to daylength, as larger gametophytes developed under long- than under short-days. Similarly, long daylengths were optimal for gametophyte growth in other kelp or kelp-like species (*Ecklonia radiata*: Moring et al. 2013; *Undaria pinnatifida*: Choi et al. 2005; *Desmarestia ligulata*: Edwards 2000). Our gametophytes originated from Helgoland (North Sea), where the seawater temperature regularly reaches 18°C in summer (Bartsch et al. 2013). Optimal gametophyte growth (10°C–18°C and simulated summer long days) in the present study was thereby consistent with the environmental conditions that the species encounters at Helgoland during late spring to summer. In contrast, under simulated winter photoperiods and low temperatures of 5°C, gametophyte growth was impaired.

As growth and fertility are often mutually exclusive processes in kelps (Lüning and Neushul 1978, Izquierdo et al. 2002), the induction of fertility showed a contrasting pattern to gametophyte growth. Gametogenesis in *L. digitata* gametophytes was readily induced between 5°C and 15°C, with very high to maximum sporophyte recruitment under simulated conditions of long photoperiods and enriched nutrient conditions, a situation that is probably only present along the Norwegian coastline in summer (Wassmann and Aadnesen 1984) or when coastal eutrophication is encountered (Norderhaug et al. 2015). The optimum temperature range for gametogenesis of *L. digitata* reported here is similar to previous studies with Helgoland material that reported high fertility between 5°C and 12°C, and slightly reduced fertility at 17°C (tom

Dieck 1992, Müller et al. 2008). Although gametogenesis was also observed at 18°C here, there was essentially no sporophyte recruitment (<1%). This agrees with earlier observations in which gametogenesis in *L. digitata* became fully inhibited between >17°C and 21°C (tom Dieck 1992). The ecological consequences of relatively high summer temperatures above 17°C will probably be to considerably reduce immediate sporophyte recruitment, while potentially enhancing recruitment in autumn to spring as the onset of vegetative gametophyte growth will multiply the number of potentially reproductive gametophyte cells.

Interestingly, there was a mismatch between fastest development of gametogenesis, which took place at 10°C–15°C under long days and enriched nutrient conditions, and best recruitment conditions for juvenile sporophytes. As the overall density of normal sporophytes and partheno-sporophytes was also greatest at 10°C, this indicated that rates of oogenesis were highest at this temperature. In contrast, the greatest number of normal sporophytes developed at 5°C, long day and high nutrient conditions, where gametogenesis was much slower. It is thus assumed that fertilisation was probably hampered at 10°C. In culture, it may be observed that unfertilised eggs of Laminariales develop parthenogenetically, resulting in irregular-shaped partheno-sporophytes without clear polar development and often without formation of rhizoids (e.g. Motomura and Sakai 1981, tom Dieck 1992); in some cases adult, fertile partheno-sporophytes with normal morphology may develop (Fang et al. 1978, Ar Gall et al. 1996). Egg release in kelps occurs in the night during the 1st h after onset of darkness (Lüning 1981, Li et al. 2013) and released eggs secrete the universal pheromone lamoxirene that mediates spermatozoid release from antheridia and their attraction to the eggs (Lüning and Müller 1978). If gametogenesis is very rapid and many eggs are released at the same time, theoretically all spermatozooids may be released during the first massive egg extrusion event, as males of many kelp species are protandric (fertile before the females; Hsiao and Druehl 1971, Bartsch pers. obs.). As eggs are released over a series of days (Lüning 1981), the subsequent eggs might fail to be fertilised and develop parthenogenetically, particularly as the life-span of sperm is generally no more than 12 h (Li et al. 2013). In this study, there may not have been enough sperm to fertilise all eggs due to a potential mismatch in the initial proportion of female:male gametophytes (~77:23, although gametophytes had been derived from freshly released zoospores approx. 6 months earlier). The effect of this possible mismatch was, however, only pronounced at 10°C where overall egg formation was fast and highest.

It is not known whether this observation may also be relevant in the field.

In total, long photoperiods combined with enriched nutrient conditions in cooler temperatures optimally integrated the development of all ontogenetic stages, namely oogonia development, egg formation and sporophyte recruitment. While enriched nutrient conditions also favour reproduction and recruitment in other kelp species (e.g. Hsiao and Druehl 1973, Edwards 2000, Carney and Edwards 2010), optimum daylength conditions for gametogenesis and recruitment might be species-specific (tom Dieck 1989, Wiencke and Clayton 1990, Wiencke et al. 1996, Choi et al. 2005, Roleda 2016).

This study underpins the results of earlier studies that showed that life cycle processes in kelps have different thermal optima, even if they are short and transient – a property that probably drives the seasonal cycle. In *L. digitata* from Helgoland, gametogenesis was fastest at 15°C but sporophyte recruitment was optimal at 5°C. This relationship may possibly vary between populations or among latitudes but evidence is still incomplete. Müller et al. (2008) showed that the optimal temperature requirements for *L. digitata* zoospore germination from Helgoland and Spitsbergen differed from those for egg and young sporophyte formation, and the latter (Arctic) had generally lower temperature optima. It has been previously suggested that *L. digitata* gametophytes develop vegetatively in summer and autumn directly after release of zoospores under high seawater temperatures and that new microscopic sporophytes develop before winter (Sjøtun and Schoschina 2002), which is supported by our results.

If optimum reproductive temperatures are reached later in the year, as is likely under global warming scenarios, then development of new sporophytes will possibly start later. This may have large implications for several ecosystem functions such as productivity, species interactions and community structure (Lotze and Worm 2002). Additionally, if climate change causes reduced nutrient levels and/or increasing temperatures in summer (>18°C), southern recruitment will be drastically hampered or even prevented. In contrast, under global change scenarios for middle Europe, increase of winter temperatures from 5°C to 10°C under otherwise stable high nutrient and short day conditions, will probably not hamper winter recruitment of *L. digitata*. These assumptions do not take into account that local populations may have adapted to their respective conditions (e.g. Mohring et al. 2014) and thus may show a different response to multifactorial changes than just one population. Furthermore the current investigation did not verify the situation in the field or consider

spores taken throughout the season, but it clearly shows that responses of kelp life stages vary in time.

Overall, a high buffer capacity was evident enabling recruitment, albeit with different success, over a broad combination of the tested environmental factors. Interestingly our data show that recruitment in summer is possibly prevented over a wide latitudinal scale – although due to different reasons. Summer conditions near the southern distribution limit may theoretically cause several consecutive responses: (1) They may potentially enhance recruitment at a later stage as released summer zoospores will grow vegetatively after germination and thereby multiply the number of potential reproductive cells. (2) As high summer temperatures (>17°C) delay gametogenesis and recruitment, the potential recruitment period is shifted to cooler autumn to winter conditions. (3) As all vegetative gametophyte cells are omnipotent and may become fertile later when conditions become cooler again, zoospores released under unfavourable summer conditions are not necessarily lost from the population, as described by Bartsch et al. (2013). This sequence of events would prevent the formation of juvenile sporophytes in stressful summer conditions (low nutrients, high temperatures and high irradiation) but nevertheless ensures recruitment later in the year.

The recruitment situation certainly is very different near the northern distribution limit of the species in northern Norway or the Arctic (Lüning 1990) where, in spring to summer, long daylengths coincide with low water temperatures around 5°C–10°C (e.g. Wassmann and Aadnesen 1984, Hop et al. 2002, Bartsch et al. 2016). Our results suggest a high recruitment capacity especially in spring during increasing daylength but only as long as nutrient conditions are still sufficient. In the Arctic, primary nutrients may be near zero after the spring bloom (Hodal et al. 2012) and thereby gametogenesis and recruitment over summer in the Arctic may also be delayed by insufficient nutrients, despite optimal temperatures.

In conclusion, our results highlight the importance of understanding the environmental drivers for growth and reproduction of all life-cycle stages of kelps in order to better understand the performance of these ecosystem-engineering species in the context of global change. *In situ* field studies over broader geographical scales investigating the implications of this study for different populations and with zoospores derived over the season are urgently needed.

Acknowledgements: This work was supported by a STSM Grant from the COST Action “Phycomorph” FA1406 and by the Portuguese Science Foundation (FCT) programs EXCL/

AAG-GLO/0661/2012, UID/Multi/04326/2013, BiodiVERsA Biodiversa/0004/2015 and PTDC/MAR-EST/6053/2014. We thank A. Wagner for collecting and maintaining the algal material and for help with laboratory facilities. We thank the three reviewers for their helpful suggestions.

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Bionotes



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Gareth A. Pearson (researcher at CCMAR, the Centre of Marine Sciences, Algarve, Portugal) and Ester A. Serrão (lecturer at the University of Algarve) coordinate a research team which aims to understand patterns and processes mediating marine genetic diversity, function and evolution, from ecological to deep evolutionary scales. Their research topics include causes and consequences of genetic biodiversity, population dispersal/connectivity, environmental genomics and adaptive evolution, in the context of climate and environmental change. These are studied across the diversity of marine systems ranging from microorganisms to large marine forests.



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Inka Bartsch is a senior scientist at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. During her PhD, which she obtained from the University of Hamburg in 1989, and up to 1998 she worked at the Biologische Anstalt Helgoland. Although being involved in a wide array of initiatives including monitoring and remote sensing, her major interest is in unravelling the ecology and ecophysiology of dominant brown algal key species, especially kelps, their development, recruitment success, competitive ability and productivity under major abiotic drivers in order to better understand their role in the shallow water ecosystems and their acclimation and adaptation capacities.