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Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea

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ABSTRACT: Spores and gametophytes are considered to be those life-history stages of kelps most susceptible to environmental perturbations and, in particular, to temperature and UV radiation. Microstages of Arctic (*Saccharina latissima*, *Laminaria digitata*, *Alaria esculenta* from Spitsbergen) and temperate kelp species (*S. latissima*, *L. digitata*, *L. hyperborea* from the North Sea) were exposed in 2-factorial experiments to different temperatures (2 to 18°C) and radiation conditions (photosynthetic active radiation, UV-A radiation, UV-B radiation). Our results reveal ecotypic differentiations in the stress responses of zoospores of *L. digitata* and *S. latissima* from the Arctic and North Sea. UV-A radiation either enhanced the formation of gametes at elevated temperatures in *L. digitata* or impaired egg release and subsequent sporophyte formation in *L. hyperborea*. Microstages exposed to additional UV-B radiation were strongly inhibited to a population-, species- and life phase-specific degree at suboptimal and optimal temperatures. Only gametogenesis of *Laminaria* spp. was shown to be tolerant to UV-B exposure. Conclusively, in respect to a future scenario of elevated UV radiation regimes and higher summer temperatures in Arctic and North Sea waters, summer-recruiting Arctic species and *L. digitata* from the North Sea might become endangered by a frequent disturbance of their microstages.

KEY WORDS: Ecotypes · Egg release · Gametogenesis · Germination · Kelp · Sporophyte formation · Zoospores

1. INTRODUCTION

Kelps, i.e. brown algae of the order Laminariales, are important components of coastal ecosystems in polar and temperate waters, especially in the Northern Hemisphere (Lüning 1990). Biogeographic distribution of seaweeds depends on the temperature requirements for growth and reproduction, as well as on the temperature tolerance of the various stages in the life cycle of individual species (Breeman 1988, tom Dieck 1993, Wiencke et al. 1994). The distribution of kelp species is often determined by their reproductive cells as they are more vulnerable to changes in their abiotic environment, especially temperature, compared to other stages in their life histories (van den Hoek 1982, Coelho et al. 2000).

Whereas the latitudinal distribution is limited by the temperature requirements, the radiation regime, especially at short wavelengths, was found to be a major factor for determination of depth zonation of seaweeds (Hanelt 1998, Roleda et al. 2005, Wiencke et al. 2006). The microstages, in particular the zoospores of brown algae, were more susceptible to changes in the radiation conditions as compared to their macrothalli (Véliz et al. 2006). Moreover, zoospores of deep-water species typically exhibited a higher UV radiation (UVR) susceptibility than those of eulittoral species (Bischof et al. 2006).

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Hitherto, numerous studies had demonstrated the independent effects of temperature and UVR (280 to 400 nm) on physiology of various life history stages of Laminariales (reviewed in Bartsch et al. 2008, Roleda et al. 2007). In contrast, only few studies are available on the interactive effects of temperature and UVR (280 to 400 nm) on macroalgae (i.e. Gómez et al. 2001, van de Poll et al. 2002, Rautenberger & Bischof 2006). The interaction between these factors can lead to variable responses. As an example, UVR-induced photoinhibition of Photosystem II was less expressed in sporophytes of the red alga *Gelidium pulchellum* at 20°C compared to 15°C (Gómez et al. 2001) and in the green algae *Ulva clathrata* and *U. bulbosa* at 10°C compared to 0°C (Rautenberger & Bischof 2006), respectively. Repair and photoprotective mechanisms under UVR were equally stimulated by increasing temperature (Gómez et al. 2001). On the other hand, UVR effects on photosynthesis, growth and DNA were not temperature-dependent in sporophytes of the red algae *Palmaria palmata*, *Coccotylus truncatus* and *Phycodrys rubens* (van de Poll et al. 2002).

To our knowledge only 2 studies have explored multifactorial effects on the microstages of macroalgae so far (Lotze & Worm 2002, Hoffman et al. 2003). The recruit density of the green alga *Enteromorpha intestinalis* responded in a complex manner to temperature and UVR. In the absence of grazing and at low nutrient loads, UVR reduced recruitment at ambient temperatures, but enhanced recruitment at low temperatures (Lotze & Worm 2002). In a study on brown algae, Hoffman et al. (2003) showed that the inhibiting effect of UVR on spores and gametophytes of *Alaria marginata* as well on zygotes and germlings of *Fucus gardneri* was less strong at higher temperatures.

Nevertheless, our knowledge on interactive effects of temperature and UVR on macroalgae is limited, especially with respect to possible ecotypic differentiations of populations of widely distributed kelp species. The purpose of the present study was to examine the interactive effects of 4 temperatures and 3 light conditions on zoospores, gametes and oogonia of Laminariales from the Arctic (Spitsbergen) and the North Sea (Helgoland). We compared stress responses of microstages of the Arctic, cold-temperate and amphiatlantic *Saccharina latissima* and *Laminaria digitata* from Spitsbergen and Helgoland. Additionally, we studied microstages of the cold-temperate to Arctic, amphiatlantic *Alaria esculenta* and warm-temperate east-Atlantic *L. hyperborea*. The parameters studied include germination, gametogenesis, egg release and sporophyte formation.

2. MATERIALS AND METHODS

2.1. Algal material

2.1.1. Zoospores

Fertile algal material was collected in May 2006 by SCUBA diving in the Kongsfjorden close to Ny Ålesund, Spitsbergen (SP, 78° 55′ N, 11° 56′ E). *Laminaria digitata* (Huds.) Lamour. was collected at 2 to 4 m depth, *Saccharina latissima* (L.) Lane, Mayes, Druehl, Saunders and *Alaria esculenta* (L.) Grev. were collected at 4 to 6 m depth. The material from the North Sea was collected around the island of Helgoland (HLG, 54° 11′ N, 7° 53′ E); *L. digitata*, at 2 to 4 m depth (August 2005); and *S. latissima* and *Laminaria hyperborea* (Gunn.) Fosl., both at 4 to 6 m depth (November 2005). Blades of ≥5 ind. were cleaned of fine sediment and potential epiphytes (e.g. ciliates, bacteria) with tissue paper. Sori were cut, dried with tissue paper and stored in dark, moist chambers at 3 ± 2°C over 1 or 2 nights. To release zoospores, individual sori were immersed in seawater (0.2 µm pore size, 10 ± 2°C, HLG sori in Provasoli-enriched seawater, PES; Starr & Zeikus 1993) under dim white light at room temperature. After ≤60 min, the sori were removed, and the released zoospores were filtered through a 20 µm gauze (Nytal HD 20, Hydro-Bios). Zoospores in suspensions were counted by use of a Neubauer chamber (Brand) under 200× magnification of an Axioplan microscope (Zeiss) and diluted to required densities. Zoospore suspensions of 3 to 5 ind. species^{-1} were separately distributed by dispensettes (Brand) into 35 × 10 mm culture dishes (Corning TM®) containing 2 cover slips each (0.8×10^2 to 3.3×10^2 zoospores mm^{-2}).

2.1.2. Gametophytes

Male (♂) and female (♀) vegetative gametophyte tufts from stock cultures of *Laminaria hyperborea* and *L. digitata* (AWI seaweed collection, HLG isolates ♂ 3155, ♀ 3156 and ♂ 3157, ♀ 3158, respectively) were gently ground with pestle and mortar into few-celled fragments, and the suspensions were diluted with PES. Gametophyte suspensions (40 ml) were allotted (Brand dispensettes) to 85 × 15 mm Petri dishes (10 to 40 gametophytes mm^{-2}) separated by gender in 5 replicates treatment^{-1}. To determine the formation of ♂ and ♀ gametangia under experimental conditions, Petri dishes were immediately exposed to experimental conditions as described below. To investigate reproductive success, gametophytes had to be precultivated under dim light (10 ± 3.3 µmol m^{-2} s^{-1} PAR, photosynthetically active radiation) at 10 ± 2°C for 8 (♂) or

15 (♀) d. After induction of fertility, 30 ml of fertile male gametophyte suspension was added to each Petri dish containing fertile female gametophytes (separated by 20 µm gauze). After fertilization of eggs in darkness for 48 h, male gametophyte fragments were excluded and PES (40 ml) was renewed.

2.2. Experimental conditions

The zoospores, the sterile gametophytes, or the gametophytes with fertilized oogonia were exposed in environmentally controlled chambers set to 2, 7, 12 and 18 ± 1.4°C, representing a broad temperature regime regularly occurring in HLG and Arctic seawaters. In the case of the zoospores of *Saccharina latissima* and *Laminaria hyperborea* from HLG, temperature was kept constant by the use of thermostats (Haake D1-8-V, DC1-V, Thermo Electron). Microstages were treated with radiation for 8 h. Therefore, culture dishes were covered with cut-off filter foils transparent to wavelengths between 400 and 700 nm (URUV Ultraphan UV farblos, Digefra), to between 320 and 700 nm (Folanorm SF-AS, Folex), or to between 295 and 700 nm (URT 140 Ultraphan UV farblos, Digefra) corresponding to 3 irradiation treatments: (1) PAR (P), (2) PAR + UV-A (PA), or (3) PAR + UV-A + UV-B (PAB). The transmittance of the cut-off filters was similar to that described by Brouwer et al. (2000). PAR (20 ± 3 µmol m^{-2} s^{-1}/6.3 W m^{-2}) was generated by fluorescent tubes (36 W, true light II Powertwist) and dimmed by black net gauze (Lüdermann). UV-A (320 to 400 nm; 5.8 W m^{-2}) and UV-B (280 to 320 nm; 0.34 W m^{-2}) was generated by UV-A-340 tubes (40 W; Q-Panel). The latter emitted a spectrum qualitatively comparable to natural solar radiation in the wavelengths 280 to 340 nm. Underneath cut-off filters, PAR irradiances were measured by a SA cosine-corrected flat-head sensor attached to a LI-COR Li-190 radiometer (LI-COR Bioscience) and spectral irradiances by cosine sensors connected to UV-VIS spectroradiometers (on HLG: Marcel-Kruse; on SP: Ramses SAM 80f6 to IPS 104, TriOS Optical Sensors). The biologically effective dose was calculated using the function for generalized plant damage action spectrum (280 to 312 nm; Caldwell et al. 1971). Light regimes at different experimental conditions are summarized in Table 1.

Table 1. Light regime at different experimental conditions. PAR: photosynthetic active radiation; UV-A: UV-A radiation (320 to 400 nm); UV-B: UV-B radiation (280 to 320 nm)

Irradiance treatment	Unweighted irradiance (W m^{-2})			Weighted irradiance (W m^{-2}) Generalized plant damage (Caldwell et al. 1971)
	PAR	UV-A	UV-B	
PAR alone (P)	5.63	0.31	0.04	0.014
PAR + UV-A (PA)	6.20	5.46	0.09	0.017
PAR + UV-A + UV-B (PAB)	6.29	5.79	0.34	0.053

2.3. Analysis

2.3.1. Germination

After exposure and 6 d of recovery in dim white light and at constant temperatures, germination rates as a percentage of germinated spores after 6 d were calculated under 200× or 400× magnification using an Axioplan microscope (Zeiss). From 300 spores $replicate^{-1}$, spores with germination tubes were counted, whilst dead and living cells without germination tubes were not differentiated.

2.3.2. Gametogenesis

Gametogenesis was estimated using an inverted microscope and by determining the percentage fertile to vegetative fragments (300 $replicate^{-1}$) (Leitz, 125× magnification) after exposure and 7 (♂) or 14 (♀) d of recovery under dim light under the same temperature conditions. Fragments were considered to be fertile if at least 1 male or female gametangium was identified.

2.3.3. Reproductive success

After exposure of fertile gametophytes to the experimental conditions and 10 d recovery under dim light and constant temperature conditions, released eggs and few-celled sporophytes were counted (at 10 mm^2) for each replicate using an inverted microscope (Leitz, 125× magnification). After 10 wk of recovery, the distribution of life stages was estimated on an area of 80 cm^2 $replicate^{-1}$ as a percentage of developed sterile gametophytes, released eggs and sporophytes.

2.3.4. Hybridization experiment

To determine ecotypic differentiations, male and female gametophyte fragments of *Saccharina latissima* (AWI seaweed collection, HLG isolates ♂ 3096, ♀ 3094; SP isolates ♂ 3123, ♀ 3124) were put together as follows: (1) SP ♀ + SP ♂, (2) SP ♀ + HLG ♂, (3) HLG ♀ + SP ♂ and (4) HLG ♀ + HLG ♂. Sporo-

phytes that developed after 4 wk of cultivation under dim white light (12 h light:12 h dark cycle; Osram lamps L 58W, Daylight) at 10°C were counted at 1 to 3 mm^2 in 5 replicates using a stereo lense (Stemi SV 6, Zeiss, 120× magnification).

2.4. Statistics

Percentage data sets were arcsine transformed. Subsequent homogeneity of variances (Cochran's test, $p < 0.01$) was achieved. In cases where data did not comply with homogeneity requirements, data were log or sine transformed. Effects of irradiation and temperature were estimated by 2-factorial, Model I ANOVA ($p < 0.05$). Post hoc multiple mean comparisons were accomplished by Tukey procedure ($p < 0.05$, honestly significance difference—HSD). The hybridization experiment was tested for multiple differences among pairs of means with *t*-test ($p < 0.05$). Statistical analyses were performed by Statistica software v.7 (StatSoft) in accordance with Sokal & Rohlf (1995).

3. RESULTS

3.1. Germination of zoospores

Zoospores of *Saccharina latissima*, *Laminaria digitata* and *Alaria esculenta* from SP germinated optimally at temperatures between 2 and 12°C and P treatments (controls) (Fig. 1a–c, Tables 2 & 3). At these temperatures, 60 to 95% of controls of *L. digitata* and *A. esculenta* germinated. However, *S. latissima* from SP exhibited an exceptionally low germination rate in controls of 8 to 35% between 2 and 12°C. Exposure to 18°C impaired germination in Arctic species completely. In contrast to the Arctic population, HLG *S. latissima* exhibited a germination rate of 85 to 92% over the whole temperature range (Fig. 1d). A temperature span of 7 to 18°C was most suitable for zoospores of *L. digitata* from HLG exhibiting 60 to 90% germination in controls, while 2°C was suboptimal for *L. digitata* with only 10% germination in controls (Fig. 1e). Optimal germination of *L. hyperborea* zoospores of 40 to 70% was observed at 7 to 12°C, while in controls at 2 and 18°C only 10 or 25% germination was achieved (Fig. 1f).

PA-treated zoospores mostly attained germination rates similar to the controls. Even a slight tendency to enhanced germination was detected after PA treatment in most species. However, this increase was statistically significant only in PA-treated *Saccharina latissima* from HLG at 12°C (Tukey HSD test, p =

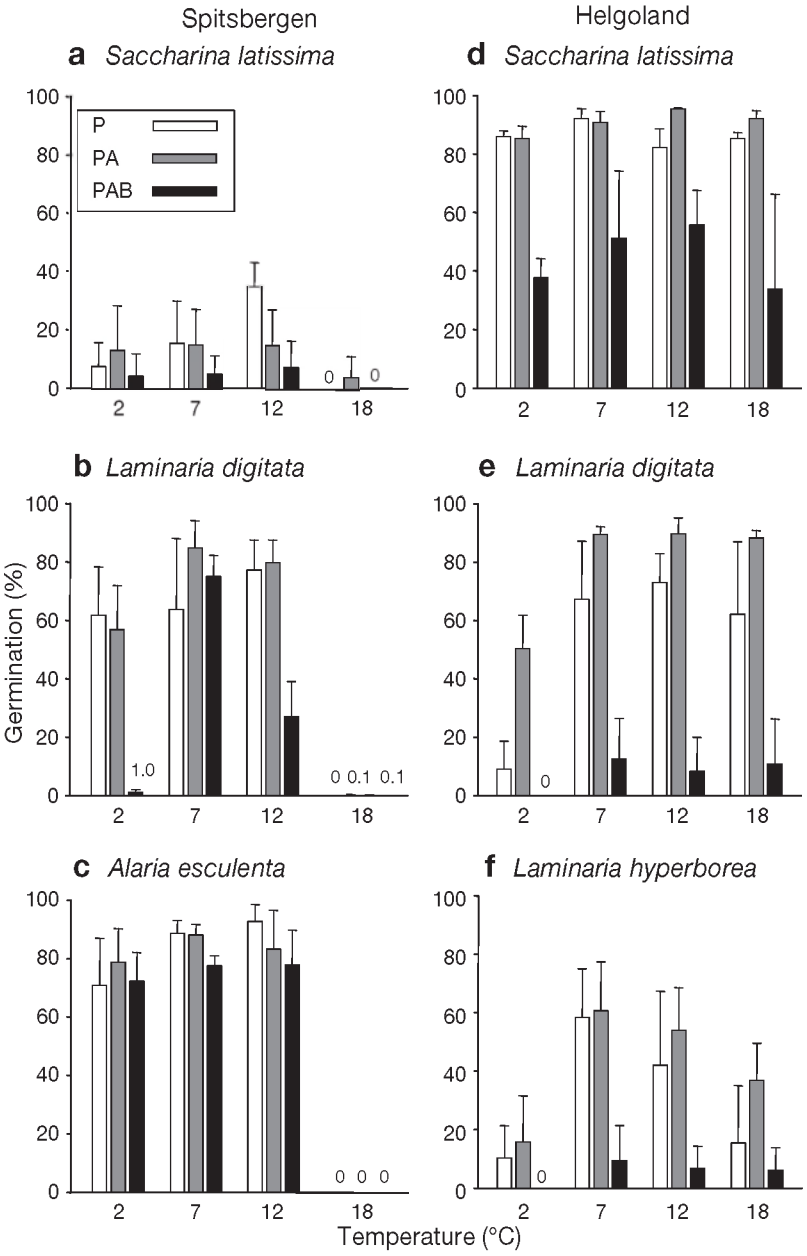

Fig. 1. Germination of zoospores of: (a) *Saccharina latissima*, (b) *Laminaria digitata* and (c) *Alaria esculenta*) from Arctic SP and (d) *S. latissima*, (e) *L. digitata* and (f) *L. hyperborea* from temperate HLG. Effects of 4 temperatures and 3 radiation treatments on germination of zoospores (mean + SD), examined after 8 h exposure and 6 d of recovery under dim white light (PAR) at constant temperatures. White bars: PAR (P), grey bars: PAR + UV-A (PA), black bars: PAR + UV-A + UV-B (PAB)

0.017). In contrast, a significant inhibition of germination after PA treatment was evident only in zoospores of *S. latissima* from SP at 12°C (Tukey HSD test, $p \leq 0.01$).

Upon exposure to PAB, *Saccharina latissima* from SP showed a temperature-dependent inhibition of 74 to 90% compared to the controls (Fig. 1, Table 2). At both low and high temperatures, zoospores of *Laminaria digitata* from SP were inhibited to 65% (or rather, 98% of those of the control), while at 7°C even higher germination compared to controls was obtained (Table 2). The most UV-B-tolerant species tested was *Alaria esculenta*, with significant differences in germination only between the P and PAB treatments at 12°C (Tukey HSD test, $p = 0.047$). This species was, moreover, the only species without discerned interactive effects of temperature and irradiation (Table 3).

PAB-treated zoospores of *Saccharina latissima* from HLG achieved 39 to 63% germination at all tested temperatures, and UV-B effects were only slightly temperature dependent. However, stronger UV-B inhibition was observed in *Laminaria* spp. Germination of HLG *L. digitata* and *L. hyperborea* was reduced upon PAB treatment to approximately 10% germination at 7 to 18°C and completely inhibited at 2°C.

3.2. Gametogenesis

After exposure to P, 20 to 35% of the male *Laminaria digitata* gametophytes from HLG produced antheridia and 22 to 40% of the females produced oogonia (Fig. 2a,b). The formation of gametangia was temperature dependent in both sexes of *L. digitata*; in particular, more antheridia and less oogonia developed at elevated temperatures after exposure to P or PAB (Fig. 2a,b, Table 3). Additionally, gametogenesis of *L. digitata* was not negatively affected by PAB treatments compared to controls and showed the same temperature-dependent pattern as the controls. However, a significant enhancement of gametogenesis was found in PA treatments at 7 to 18°C. While at 2°C, no PA enhancement was evident, formation of antheridia and oogonia of PA-treated

Table 2. UV-B inhibition (%) of germination and egg release compared to P treatments at 4 temperatures. ng: no germination in controls

Process	Species	Location	2°C	7°C	12°C	18°C
Germination	*Saccharina latissima*	SP	84.4	73.9	90.1	ng
	Laminaria digitata		98.3	–17.6	65.1	ng
	Alaria esculenta		–1.9	12.6	16.1	ng
	S. latissima	HLG	56.2	44.3	37.6	60.3
	L. digitata		99.9	81.3	88.7	82.6
	L. hyperborea		99.9	83.7	88.7	60.3
Egg release	*L. digitata*	HLG	7.4	38.6	94.0	40.0
	L. hyperborea		21.4	81.4	87.0	6.0

Table 3. Analysis of irradiance and temperature effects (2-way ANOVA, Tukey HSD Test, n = 5) on germination, gametogenesis and egg release of Laminariales species. *Significant, ns: not significant, ♂: male; ♀: female

Dependent variable Species, location	Source of variation	df	*F*	p	
Germination					
Saccharina latissima[a]	Irradiance (A)	2	19.29	0.000	*
SP	Temperature (B)	3	26.23	0.000	*
	A × B	6	11.37	0.000	*
Laminaria digitata	A	2	66.34	0.000	*
SP	B	3	259.10	0.000	*
	A × B	6	22.45	0.000	*
Alaria esculenta	A	2	4.89	0.014	*
SP	B	3	191.02	0.000	*
	A × B	6	2.04	0.078	ns
S. latissima	A	2	103.28	0.000	*
HLG	B	3	30.49	0.000	*
	A × B	6	6.16	0.000	*
L. digitata	A	2	115.03	0.000	*
HLG	B	3	27.92	0.000	*
	A × B	6	3.52	0.006	*
L. hyperborea	A	2	28.31	0.000	*
HLG	B	3	14.97	0.000	*
	A × B	6	2.64	0.027	*
Gametogenesis					
L. digitata (♂)	A	2	82.37	0.000	*
HLG	B	3	37.95	0.000	*
	A × B	6	11.04	0.000	*
L. digitata (♀)	A	2	60.22	0.000	*
HLG	B	3	4.16	0.011	*
	A × B	6	9.96	0.000	*
L. hyperborea (♂)	A	2	6.08	0.004	*
HLG	B	3	0.61	0.611	ns
	A × B	6	2.32	0.048	*
L. hyperborea (♀)	A	2	0.46	0.634	ns
HLG	B	3	4.23	0.010	*
	A × B	6	4.48	0.001	*
Egg release					
L. digitata	A	2	64.99	0.000	*
HLG	B	3	59.65	0.000	*
	A × B	6	27.47	0.000	*
L. hyperborea	A	2	28.79	0.000	*
HLG	B	3	61.05	0.000	*
	A × B	6	9.71	0.000	*

[a]n = 3

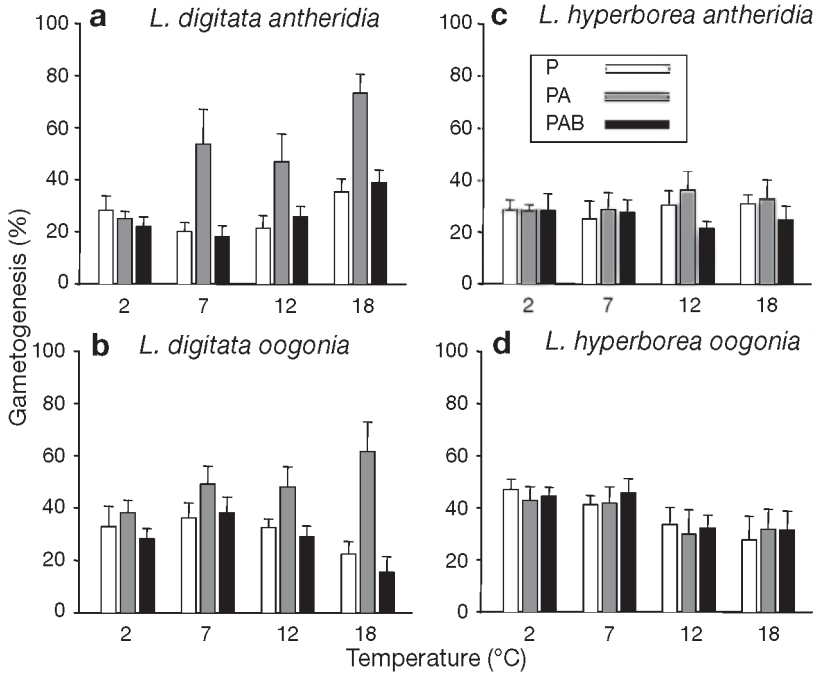

Fig. 2. Gametogenesis of: (a) female and (b) male *Laminaria digitata* as well as (c) female and (d) male *L. hyperborea* from HLG. Effects of 4 temperatures and 3 radiation treatments on gametogenesis (mean + SD) after 8 h exposure and 7 (♂) or 14 (♀) d recovery under dim white light at constant temperatures

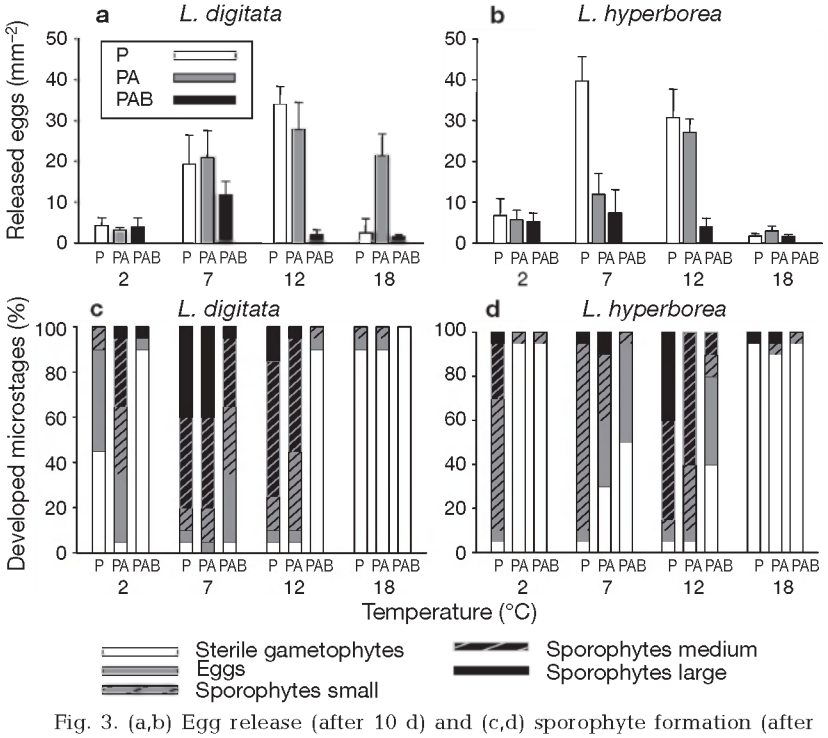

Fig. 3. (a,b) Egg release (after 10 d) and (c,d) sporophyte formation (after 10 wk) of: (a,c) *Laminaria digitata* and (b,d) *L. hyperborea* from HLG. Effects on egg release and sporophyte formation after 8 h exposure to 4 temperatures and 3 radiation treatments and 10 d or 10 wk of recovery under dim white light (PAR) at constant temperatures. Sporophytes ≤4 cells are designated small; ≥5 and ≤14 cells, medium; and ≥15 cells, large

gametophytes increased with increasing temperatures to a maximum of 73% (♀) or rather 80% (♂) at 18°C.

A total of 25 to 37% of *Laminaria hyperborea* gametophytes developed antheridia or 27 to 47% oogonia in all treatments. There were only very few interactive effects of UVR and temperature on antheridia and oogonia formation (Table 3). Whereas significantly more oogonia were formed in *L. hyperborea* under the P treatment at 7 and 12°C compared to the PA treatment at 18°C ($p \leq 0.01$), male gametogenesis differed significantly only between PA and PAB treatments at 12°C ($p \leq 0.01$).

3.3. Egg release

After 10 d of recovery under dim white light, oogonia of *Laminaria digitata* released 19 to 34 eggs mm^{-2} in controls at 7 to 12°C and 20 to 28 eggs mm^{-2} in PA treatments at 7 to 18°C, respectively (Fig. 3a). After PAB exposure, gametophytes formed only 11.8 eggs mm^{-2} at 7°C and only 1.5 to 4 eggs mm^{-2} at other temperatures. Only 2.5 to 4.3 eggs mm^{-2} developed in controls at 18°C and in all light treatments at 2°C. After 10 wk of recovery, a UV- and temperature-dependent response pattern corresponding to the UV- and temperature-dependent fertility pattern became obvious in the distribution of life stages. This was apparent in the formation of sporophytes under optimal conditions or sterile gametophytes under suboptimal conditions (Fig. 3c). However, mostly sterile gametophytes were observed after exposure to PA at 18°C, although egg release was enhanced under these conditions. On the other hand, sporophytes developed in PA-treated gametangia at 2°C.

The largest amounts of eggs of *Laminaria hyperborea* were released from controls at 7 to 12°C, with about 30 to 45 eggs mm^{-2}, and in the PA treatment at 12°C, with 27 eggs mm^{-2} (Fig. 3b). The release of eggs was reduced after PA and PAB exposure at 12°C to 7.4–12 eggs mm^{-2}, as well as after PAB treatment at 7°C to 4 eggs mm^{-2} ($p \leq 0.01$). At both low and high temperatures, no irradiance effect was found and only 1.6 to 6.7 eggs mm^{-2} were liberated. After 10 wk of recovery, similar UV- and temperature-dependent response patterns were apparent as

found for egg release (Fig. 3d). The only exception was the control of *L. hyperborea* at 2°C, where numerous sporophytes developed, although egg release was similarly low under the 3 radiation conditions at this temperature and only very few sporophytes developed after exposure to P and PAB.

3.4. Hybridization experiment

Male and female isolates of *Saccharina latissima* from SP produced approximately 260 sporophytes mm^{-2}. The combination of SP females with HLG males resulted in significantly different patterns of sporophyte formation ($p \leq 0.001$). From the latter combination of gametophytes, only half the number of sporophytes (114 sporophytes mm^{-2}) developed. HLG females produced close to 20 sporophytes mm^{-2}, with both males from SP or HLG, and were significantly disparate from Arctic females ($p \leq 0.001$), but did not differ among themselves ($p = 0.87$). In none of the combinations were amorphous parthenogenetic sporophytes detected.

4. DISCUSSION

One main result of the present study is the existence of a 2-factorial ecotypic differentiation in zoospores of Arctic *Saccharina latissima* and *Laminaria digitata* that are interactively affected by UVR and temperature. Crossing experiments between *S. latissima* from SP and HLG support the hypothesis of ecotypic differentiation and indicate that the populations of *S. latissima* from both locations have to be regarded as varieties if not as subspecies of 1 species. The second important finding is that gametogenesis of *L. digitata* is enhanced by UV-A, especially at elevated temperatures. Moreover, the germination of zoospores of various species was elevated after exposure to UV-A. In contrast, in *L. hyperborea*, the release of eggs and subsequent sporophyte formation was inhibited by UV-A. The tested microstages of the various species were impaired by UV-B to differential degrees, depending on temperatures. Exclusively, germination of *Alaria esculenta* and gametogenesis of *L. digitata* and *L. hyperborea* were completely tolerant against UV-B.

4.1. Thermal ecotypes in Laminariales from Arctic and cold-temperate waters

Acquired thermal responses of Arctic zoospores (controls) evinced a strong adaptation to their polar environment. Likewise, zoospores germinated well up to 12°C (Fig. 1), while 6°C is a typical summer temperature for surface waters from SP (Wiencke et al. 2007a). But, in contrast to the studied temperate populations, germination of Arctic zoospores was completely inhibited at 18°C (Fig. 1). This extends the theory of algal adaptation to polar temperatures: in the Laminariales this concept was hitherto solely based on the temperature requirements of sporophytes and the sexual reproduction in the gametophytes (Wiencke et al. 1994). According to this hypothesis, seaweeds expanding their geographic distribution towards higher latitudes initially increase their tolerance to low temperatures, subsequently lowering their lower survival limit and optimizing growth and reproduction at low temperatures to survive in polar environments. Simultaneously, the normal upper temperature limits of reproduction and growth (which is ≥15 to 20°C for temperate Laminariales) decreases, resulting in death at these higher temperatures (Wiencke et al. 1994). Therefore, due to the large spatial separation of HLG and SP, we postulate a differentiation of investigated Arctic and temperate *Saccharina latissima* and *Laminaria digitata* into thermal ecotypes.

Temperature- and light-related ecotypes were earlier reported for less separated gametophytes and sporophytes of *Saccharina latissima* from Long Island Sound (USA) and the Atlantic coast of Maine (USA) by Gerard (1990) and Bruhn & Gerard (1996). Conformingly, Lüning (1975) and Lüning et al. (1978) observed differences in physiological characteristics of sporophytes of *S. latissima* from HLG and the Isle of Man (British Isles) as only populations from the British Isles died at temperatures above 16°C. Admittedly, Bolton & Lüning (1982) did not find ecotypic differentiations in thermal responses within gametophytes of *S. latissima* isolates from France, Brittany, Norway, or HLG or from *L. digitata* isolates from Canada and HLG. But investigations by Billot et al. (2003) found genetic differentiations in continuous, non-fragmented forests of *L. digitata* at distances greater than only 10 km. The different capacities for sporophyte formation of *S. latissima* demonstrated here through crossing experiments between Arctic and HLG isolates support additionally an ecotypic differentiation in *S. latissima* (Fig. 4). However, distinct morphologies of *S. latissima* blades with or without bullae between populations from the Isle of Man and HLG (Lüning 1975, Lüning et al. 1978), as well as between SP and HLG (authors' pers. obs.), also prompt us to classify at least *S. latissima* from SP and HLG as varieties if not even subspecies.

4.2. Reproductive success of Laminariales under changing temperature and UVR regimes

Similar to Arctic zoospores the tested HLG laminarian microstages are thermally well adapted to their environment and their recruiting seasons, respectively.

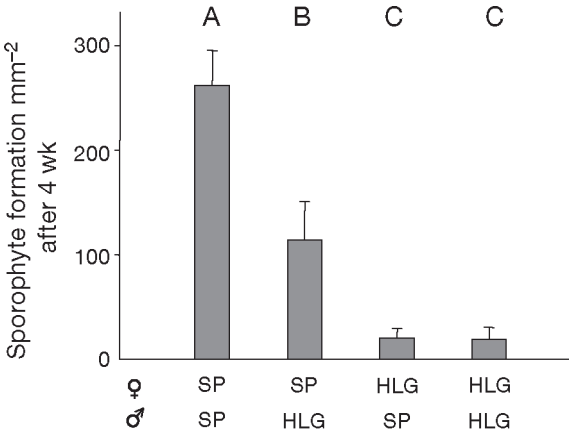

Fig. 4. *Saccharina latissima*. Crossing experiment between populations from the Arctic (Spitsbergen) and the North Sea (HLG). Letters above bars denote significant differences (*t*-test, $p < 0.05$). ♀: female gametophytes; ♂: male gametophytes. Experimental temperature: 10°C

For example, *Laminaria digitata* from HLG releases its zoospores in summer and autumn, concomitant to high seawater temperatures (15 to 20°C), while eggs and the juvenile sporophytes develop in winter and spring at low temperatures (4 to 12°C; Wiltshire 2004) (Lüning 1980). In this respect, the optimal temperature requirements of *L. digitata* zoospores differed from those of its eggs and young sporophytes at 18°C (Figs. 1 & 3). Zoospore germination, egg release and sporophyte formation of *L. hyperborea* from HLG exhibited an optimum at 7 to 12°C (Figs. 1 & 3), which is consistent with the temperature range the species encounters during recruitment in winter and early spring. The strong inhibition of germination in both HLG *Laminaria* species at 2°C, as an unusual temperature for HLG (www.bsh.de), corresponds to the results of Sjoetun & Schoschina (2002), who found primary cell development of spores of Norwegian *L. digitata* and *L. hyperborea* to be very slow at 0°C. Nevertheless, controls of *Saccharina latissima* zoospores from SP and HLG were not inhibited at 2°C in the present study, nor did embryospores of Norwegian *S. latissima* develop rapidly at 0°C (Sjoetun & Schoschina 2002). Further, Sjoetun & Schoschina (2002) suggested that *S. latissima* is well adapted to Arctic conditions.

Gametophytes of *Saccharina latissima* are usually eurythermal (Bolton & Lüning 1982), and this also applies to the germination of zoospores of *S. latissima* from HLG (Fig. 1). Furthermore, a high reproductive plasticity in thermal adaptation of germination was found for *S. latissima*, as monthly variations were observed as a function of seawater temperature and PAR. Similar responses with respect to primary cell enlargement and female gametophytic growth were found in *S. latissima* from Long Island Sound (USA; Lee & Brinkhuis 1988). Similarly, sporophytes of *S. latissima* are able to recover rapidly from exposure to high temperature (Gerard 1997) by an increase in cellular levels of Photosystem II-associated fucoxanthin-chlorophyll binding protein, as well as in the photosynthetic pigments fucoxanthin, β-carotin, chlorophyll *a* and *c* and the activity of the key Calvin-cycle enzyme RUBISCO (Davison 1987, Machalek et al. 1996).

Sporophytes of *Laminaria digitata* from Ireland showed an amplified pigment accumulation and a higher photosynthetic capacity with increasing temperature after exposure to additional blue light (380 to 500 nm) (Dring 1989). Photomorphogenetic responses to blue light, including UV-A, were also reported for gametogenesis, sporophyte formation and photoreactivation in sporophytes of various Laminariales (Lüning & Dring 1972, Lüning 1981, Dring 1989, Han & Kain 1992, 1993, Ødegaard et al. 1998). Moreover, gametogenesis of *L. digitata* was enhanced after UV-A exposure and increased with increasing temperatures (Fig. 2a,b). Additionally, a slight temperature-dependent UV-A improvement of germination of zoospores and release of eggs of *Saccharina latissima* and *Laminaria* spp. was detected in our study for the first time. A comparable UV-A/blue light–induced photoreactivation of spore germination was found in the green alga *Ulva pertusa* (Han et al. 2004).

In contrast, UV-A impaired egg release and sporophyte formation of *Laminaria hyperborea* at favorable temperatures (Fig. 3), as well as zoospore germination of Laminariales in field experiments (Wiencke et al. 2006) and photosynthetic performance of sporophytes of Laminariales (Bischof et al. 1998). Gametogenesis of PA-treated *L. hyperborea* was not ameliorated (Fig. 3) because the fluence of UV-A was too low to achieve >50% fertility in PA treatments (Lüning 1980, Ødegaard et al. 1998). An interesting ambivalent UV-A effect was obtained, since egg release of *L. digitata* and *L. hyperborea* was first depressed by the PA treatment at category temperatures. In the further course of the experiment, sporophyte formation was enhanced by UV-A at 2°C, but vice versa in *L. digitata* at 18°C (Fig. 3). For either stimulation or inhibition of morphogenetic processes by UV-A, an important role might be played by the ratio of PAR:UV-A, the temperature and the timescale.

The additional impact of UV-B inhibited germination of zoospores, egg release and sporophyte formation in a temperature-dependent and persistent way (Fig. 3). In our study, the UV-B susceptibility of egg release was determined for the first time and was comparable to that of germination of zoospores. Nonetheless, in contrast to both UV-B-sensitive unicellular propagules, gametogenesis from few-celled gametophytes was entirely UV-B tolerant at all temperatures (Fig. 2). This

high UV-B tolerance of gametogenesis might be due to a too low UV-B intensity in UVR experiments: in a study on Lessoniaceae (Laminariales), biological effective doses (BED) of 2.18 kJ m^{-2} (*Lessonia nigrescens*) and 1.41 kJ m^{-2} (*L. trabeculata*) were required to inhibit zoospores and values approximately twice this magnitude (4.22 and 2.29 kJ m^{-2}) were required to inhibit their gametogenesis (Véliz et al. 2006). Furthermore, Dring et al. (1996) and Véliz et al. (2006) reported an increase of UV-B tolerance from gametophytes to juvenile and adult sporophytes.

The most sensitive part of the life cycle of brown macroalgae—germination of zoospores—was impaired by UV-B in *Laminaria hyperborea*, *L. digitata* (HLG > SP), *Saccharina latissima* (Sp > HLG) and *Alaria esculenta*, in descending order (Table 2). Conformingly to UV-B susceptibilities of Arctic zoospores at 12°C (Table 2), similar results were obtained in laboratory UV experiments at 10°C (Wiencke et al. 2000, 2004, Roleda et al. 2006), as well as in field exposures at fjord temperature (Wiencke et al. 2006). However, observed differences in UV-B susceptibilities of zoospores from HLG species do not correspond with those noted by Roleda et al. (2005), who reported that *L. digitata* is the most UVR-tolerant species regarding germination, photosynthesis and DNA damage of zoospores at 10°C. Moreover, Dring et al. (1996) did not find any differences in UVR susceptibility of spore germination between the 3 HLG Laminariales at 10°C. The reasons for the discrepancies between studies are not clear.

However, UV-B susceptibilities of zoospores depended strongly on the experimental temperatures—e.g. UV-B stress was intensified at 2°C in *Saccharina latissima* and in *Laminaria* spp. from both locations (Table 2). Likewise higher detrimental UV-B effects at lower temperatures were observed in studies with unicellular propagules and sporophytes of red, brown and green algae, regarding recruitment, photosynthetic performance and DNA damage (Pakker et al. 2000, Gómez et al. 2001, Altamirano et al. 2003, Hoffmann et al. 2003, Rautenberger & Bischof 2006). Whilst UV-B-induced DNA damage appears to be independent of temperature, enzymatic DNA repair is more effective at higher temperatures (Pakker et al. 2000). Thus, incomplete DNA repair may reduce reproductive processes at low temperatures.

On the other hand, detrimental UV-B effects were amplified at high temperatures in zoospores of *Saccharina latissima* from both locations and of Arctic *Laminaria digitata* (Table 2), as previously reported for recruiting propagules of *Enteromorpha intestinalis* (Lotze & Worm 2002). Altamirano et al. (2003) assumed an inefficiency of certain repair mechanisms at higher temperatures, as germlings of *Fucus serratus* survived high doses of UV-B combined with low temperature, but in combination with high temperature they died. Nevertheless, detrimental UV-B effects can also occur at optimal temperatures, as shown in zoospores of *Alaria esculenta*, as well as egg release and sporophyte formation of *L. digitata* at 12°C and of *L. hyperborea* at 7 to 12°C, respectively (Table 2). In conclusion, our results imply species- and microstage-specific physiological characteristics of the damage–repair balance at various temperatures.

4.3. Implications for kelp development in an era of climate change

Clearly, the PAR level is far too low compared to the high light conditions in the field. However, as field experiments indicate, high PAR values do not inhibit germination of kelp spores in the field (Wiencke et al. 2006). In parallel laboratory experiments under dim white light, the same spore material germinated with similar rates as in the field (Wiencke et al. 2006). Another important difference is the much higher UV-B radiation in the laboratory compared with field conditions. In the field, UV-B doses recorded over various time intervals were about 10 times lower compared with common laboratory exposures (Wiencke et al. 2004). This may explain the normally lower germination rate under PAB compared to PA conditions (Wiencke et al. 2004). A similar effect has not been found in the field (Wiencke et al. 2006). These limitations have to be kept in mind when making conclusions about the performance of kelp zoospores in the field when using data obtained in the laboratory.

Certainly, Arctic and temperate marine environments will change in the near future (Bischof et al. 2006, Wiencke et al. 2007a). In the worst case scenario, IPCC (2007) forecasted an air temperature increase of approximately 4 to 6°C in the Arctic for the next century and an ozone depletion of up to 20% over the Arctic till 2020 (WMO 2007). As a consequence of higher seawater temperatures, the time window for reproduction in the short Arctic summers might become narrower and an additional increase of incident UVR may impair at least the recruitment of *Saccharina latissima* and *Laminaria digitata* by up to 65–90% (Table 2). Although zoospores of *Alaria esculenta* were UVR tolerant in this study, *Alaria* also became strongly inhibited by repeated UV-B exposure of 4 h over 3 d (Hoffman et al. 2003) or by higher PAR and UV-B irradiances (Wiencke et al. 2007b).

HLG algal recruitment, especially of summer recruiting *Laminaria digitata*, is already at its upper limit, due to a generally high impact of UVR and high summer temperatures. The seawater temperature around HLG regularly rises up to 18°C in summer (www.bsh.de),

whilst a further temperature increase of 1.5 to 2.5°C is still expected until 2100 (IPCC 2007). Gametophytes as the most temperature-tolerant microstages will reach their upper lethal limits at 21 to 22°C (HLG *L. hyperborea*), 22 to 23°C (HLG *L. digitata*), or 22 to 24°C (HLG *Saccharina latissima*) (Bolton & Lüning 1982, tom Dieck 1993). But with longer periods of exposure to high temperatures, the tolerance to high temperatures decreases (tom Dieck 1993). Additionally, since vortex oscillation has supplied more clear oceanic water into the North Sea during the last 40 yr than before this, transmission of UVR in HLG waters has simultaneously increased (Wiltshire & Manly 2004). A higher *in situ* irradiance of UV-B combined with high summer temperatures is likely to inhibit the reproductive success of HLG Laminariales by about 60 to 82% (Table 2).

An adaptation of microstages to such rapid changes of the environment is questionable. In the future, Arctic and temperate kelp forests may become endangered by the frequent inhibition of recruiting microstages at sublethal temperatures and high solar irradiation in summer and autumn. Nevertheless, the results obtained in this and other studies demonstrate that further multifactorial and field experiments are urgently needed to draw final ecological conclusions for the future development of kelp communities.

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