Daily and seasonal changes of photobiological responses in floating bull kelp

_Durvillaea antarctica_ (Chamisso) Hariot (Fucales: Phaeophyceae)

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**Abstract:** Floating seaweeds are important dispersal vehicles, especially for organisms with limited movement capacities and for the seaweeds themselves. The persistence of floating seaweeds is determined by the balance between their acclimation potential and the environmental pressures at the sea surface. Solar radiation is the most important inducer of physiological stress, varying in intensity throughout the day and the year. Therefore photoinhibition and subsequent recovery can change depending on the daily radiation dose and season. The bull kelp _Durvillaea antarctica_ is one of the most common floating seaweeds in the southern oceans, including New Zealand, Chile, and most subantarctic islands. Herein, daily cycles of maximum quantum yield (Fv/Фm), photoinhibition and recovery levels were examined in microcosm experiments with floating _D. antarctica_ throughout the year, focusing on the blade side exposed to solar radiation (sunny vs shadow side). Also, the effect of simulated wave action (blade turnover) and ultraviolet radiation (UVR) on photoinhibition and recovery of Fv/Фm was evaluated. Significant differences in maximum quantum yield were observed between blade sides, with lowest values on the sun-exposed side, especially during noontime and spring/summer months. Phlorotannins and pigments were measured during seasons with the most intense solar radiation (late spring, early summer), when Fv/Фm values were lowest. Phlorotannin, but not pigment concentrations, differed between sunny (lower concentration) and shadow blade sides (higher concentration) and throughout the daily cycle. Both blade sides had similar photoinhibition and recovery levels when blades were constantly turned over. Absence of UVR favoured the recovery capacity of Fv/Фm in both blade sides, suggesting that the photorecovery potential of floating kelps depends on the environmental conditions that kelp rafts face at the sea surface (e.g. cloudy vs sunny days, intense seawater movement and splashing vs calm sea conditions). The results confirm that photobiological stress is more severe during summer and on continuously sun-exposed blade sides, thereby damaging the blades and suppressing the floating time of _D. antarctica_.

**Key words:** _Durvillaea antarctica_, Floating kelps, in vivo fluorescence, Photoinhibition, Recovery, Solar radiation

**Introduction**

Changes in solar radiation, including both photosynthetically active radiation (PAR) and ultraviolet radiation (UVR), can occur over short (daily) and long (seasonal) scales, affecting biochemical and physiological processes in higher plants as well as in seaweeds (Hurd _et al._ 2014). Temperature and solar radiation are generally correlated in surface seawaters, providing a synergistic effect on algal physiology and growth (Lüning & tom Dieck 1989; Bruhn & Gerard 1996; Bearham _et al._ 2013).

Dynamic photoinhibition of photosynthesis is considered an important defensive response. Photoinhibition limits the photosynthetic electron transport through photosystem II (PSII) and diverts excess excitation energy by thermal dissipation. This protects important cellular components such as D1 proteins in the photosynthetic apparatus and DNA (Powles 1984; Long _et al._ 1994). In marine habitats, daily and seasonal variations in irradiance (Häder & Figueroa 1997; Huovinen _et al._ 2006), light attenuation across vertical gradients (Hanelt _et al._ 1997; Gómez & Huovinen 2011), sediment load (Roleda & Dethleff 2011) and irregular light fluctuations due to water turbulence (Wing & Patterson 1993) are factors that influence algal photosynthetic efficiency and photoinhibition levels. Continuous movements of seaweeds by wave action produce an irregular and short-term variability of light availability across the thallus surface (i.e. light vs shading pulses) that might also reduce radiation stress, thereby enhancing algal photosynthetic performance (Dromgoole 1987; Greene & Gerard 1990; Wing & Patterson 1993; Wing _et al._ 1993). Biochemical responses such as production of secondary metabolites (in brown seaweeds, mainly phenolic compounds such as phlorotannins) can serve as photoprotective and antioxidant mechanisms, thus minimizing the photodamage caused by solar radiation (Huovinen _et al._ 2006; Cruces _et al._ 2012; Abdala-Díaz _et al._ 2014).

The lifetime of most benthic seaweeds depends on their ability to remain attached to the substratum, but some environmental conditions (e.g. storms, herbivory) or aging provoke detachment and subsequent death. Nevertheless, some seaweeds feature morphological adaptations such as gas-filled structures and tissues that allow them to float at the sea surface for periods of weeks or months before disintegrating or stranding on shores (Hobday 2000; Hernández-Carmona _et al._ 2006; Graiff _et al._ 2013; López _et al._ 2013).
including the fjord region of southern Chile (Hinojosa et al. 2006; Vandendriessche et al. 2007; Rothusler et al. 2011a, b, c; Graiff et al. 2013). High radiation and temperatures cause tissue disintegration and diminish photosynthetic capacities, thereby suppressing the floating time of buoyant seaweeds at temperate latitudes (Vandendriessche et al. 2007; Rothäusler et al. 2011a; Graiff et al. 2013).

**Durvillaea antarctica** (Chamisso) Hariot (Fucales, Phaeophyceae), the buoyant bull kelp, has an extensive geographic range in the Southern Hemisphere, including New Zealand, Chile, and most subantarctic islands (Hay 1994; Fraser et al. 2010). The blades of adult individuals of *D. antarctica* have a well-developed medullary tissue that resembles an air-filled honeycomb structure, which provides high positive buoyancy that permits it to float over long distances (Hay 1994; Fraser et al. 2010). This species is one of the most common floating seaweeds in the southern oceans (Smith 2002), including the fjord region of southern Chile (Hinojosa et al. 2010; Wichmann et al. 2010). This species is one of the most common floating seaweeds in the southern oceans (Smith 2002), including the fjord region of southern Chile (Hinojosa et al. 2010; Wichmann et al. 2012) and the continental coast of central Chile (Tala et al. 2013; López et al. 2016).

The thick leathery blades of *Durvillaea antarctica* can have a thickness of 2 to 3 cm due to the medullar tissue development that produced a thallus absorptance > 90% between 300 and 700 nm (Gómez & Huovinen 2011). This morphological and bio-optical condition isolates the sides of photosynthetic blades from each other with a minimum light penetration through the honeycomb. Thereby the two blade sides are exposed to different light conditions in the natural habitat. The high buoyancy and size of *D. antarctica* lead to a particular floating pattern wherein one blade side of the detached individual is continuously exposed to sun (i.e. direct radiation) and air with low potential for overturn. In contrast, the other side of the blade is shaded and below the sea surface, receiving indirect solar radiation by reflection and scattering through seawater. Unilateral solar stress might produce physiological damage, tissue degradation and biomass loss, mainly on one blade side, whereas the other side of the blade is less stressed and physiologically competent. Some metabolic connections through the sides of the blade can occur by sieve elements as described for Fucales (Raven 2003), whereby the metabolic production on the shadow side might provide energy required for protective responses on the sunny side of the blade. Deleterious effects are expected to occur especially during the summer months, when e.g. at 39°S the intensity of solar radiation can be up to 37 times higher than in winter (Huovinen et al. 2006). Light fluctuation due to wave movements, splashing and turnover of floating *D. antarctica* would reduce the risk of photodamage. In this situation, both sides of the blade are regularly hydrated, shaded and protected from the direct impact of solar radiation, enhancing the recovery capacity of *D. antarctica* blades in a similar way as occurs in their wave-exposed benthic habitat by the whiplash movement of the blades (Wing & Patterson 1993; Stevens et al. 2002). Thus, in areas or during periods with strong winds and waves, there should be minor photobiological damage due to the continuous movement and splash of the floating kelp blade at the sea surface.

In this study we examined the hypotheses that (1) photobiological damage of floating kelp blades is higher during the summer months than during the rest of the year (by a combined effect of high-dose radiation and temperature), and (2a) frequent turnover of blades and (2b) absence of UVR reduce photodamage by enhancing the recovery potential. To test these hypotheses we determined the daily and seasonal variation in the photosynthetic performance (maximum quantum yield $F_{v}/F_{m}$, photoinhibition and recovery of photosynthetic activity) separately on the two opposite blade sides of *Durvillaea antarctica*. During the seasons with the strongest solar radiation (late spring/early summer), the daily dynamics of pigment and phlorotannin levels were additionally determined in both blade sides.

**MATERIAL AND METHODS**

Specimens of *Durvillaea antarctica* were collected during low tide in the rocky intertidal zone of Puerto Osasco, Chile (31°25'S, 71°38’W), at the northern distribution limit of this kelp in the southeastern Pacific (Collantes et al. 2002; Fraser et al. 2010). Molecular studies had shown that the populations from central Chile (30–44°S) are genetically different from the populations of southern Chile (49–56°S) (Fraser et al. 2010), and the current study focuses on the clade from central Chile.

Every 2 mo between November 2010 and January 2012, 10 kelp individuals were collected with an average weight of 2.68 ± 1.97 kg ($\bar{x} \pm s$) and a length of 1–3 m. The thickness of the medulla in *Durvillaea antarctica* is not homogenous across the blade and depends on different factors such as wave exposure, age and blade growth (Hay 1994). To minimize the potential effects of variable blade thickness on the photoinhibition/recovery responses, similar-sized individuals with well-developed medullar tissues were selected. Collected kelps were then maintained overnight in a 2000-litre tank covered with a black plastic mesh and with a constant flow of running seawater until used in the experiments.

During each experiment the daily cycle of PAR (400–700 nm), UV-A (340–400 nm) and UV-B (290–340 nm) radiation was measured using a Li-190SA quantum sensor (LI-COR Bioscience, Lincoln, Nebraska, USA), UV3pA and UV3pB sensors (Delta-T Devices Ltd., Cambridge, UK), respectively, connected with a LI-1400 data logger (LI-COR Biosciences). The sensors were close to the experimental surface and radiation was recorded between 7:00 AM and 7:00 PM at 15-min intervals. Similar systems were used in our previous studies (Graiff et al. 2013; Tala et al. 2013). The instantaneous radiation levels were used to calculate the daily dose (kJ m$^{-2}$) for each month for the duration of each experiment. The temperature in each experimental container was recorded every 2 h with a handheld thermometer (HI93510, HANNA Instruments, Cluj, Romania).

Experiments evaluated the daily variation in maximum quantum yield of floating thallus pieces of *Durvillaea antarctica* under natural solar radiation at temperate
latitudes (30°S) in outdoor microcosms. In the experiments the samples were placed in 10 transparent plastic containers (2.1 cm × 14 cm × 8 cm deep). Microfiltered (1 μm) seawater was pumped from La Herradura Bay–Coquimbo (29°58′S, 71°21′W), and seawater was continually flowing through each container at approximately 0.9 l min⁻¹. The attenuation of light inside the plastic containers was ~20%.

In the morning [7:00 AM, Greenwich Mean Time–3:00 AM in austral summer], small pieces of kelp blades were cut for the experiments (approximately 15 cm long and 10 cm wide). Previous studies have shown that small thallus pieces of photosynthetically active tissues are suitable to determine physiological responses, especially in large marine seaweeds (e.g. Hanelt et al. 1997; Bischof et al. 1998; Rothäusler et al. 2011a; Cruces et al. 2012; Gao et al. 2017). Each blade piece was carefully cleaned with a paper towel to remove any epibionts from its surface. The two blade sides were randomly assigned as ‘sunny’ and ‘shadow’, with the sunny side always referring to the upper side exposed to direct solar radiation, and the shadow side referring to the opposite, lower side, which only received indirect radiation from the surrounding water and container walls.

For each experimental month (seasonal variation), three daily cycles of maximum quantum yield were done for consecutive days under different experimental conditions. In the first daily cycle, henceforth referred to as ‘static cycle’, the blade pieces were maintained in a static condition (n = 10), with always the same side of the blade exposed to direct and the other to indirect (reflected and scattered) solar radiation throughout the entire experiment. The static daily cycle of the maximum quantum yield (Fᵥ/Fm) was measured every hour from 8:00 AM until 8:00 PM, and at 8:00 AM of the subsequent day to measure the recovery capacity.

In the second daily cycle, referred to as ‘turnover cycle’, the effect of wave agitation and occasional tumbling on the photosynthetic responses of the kelp pieces was evaluated. Blade pieces that were regularly turned over were compared with those that were maintained in static conditions (identical to the static cycle). After 1 h of exposure to solar radiation of each blade piece (n = 10) in initial static conditions (between 8:00 AM and 9:00 AM), measurements of Fᵥ/Fm were taken as initial value for the sunny and shadow sides. Thereafter, in five random containers, blade pieces were turned over every 10 min, such that sunny and shadow sides were exposed alternately to direct and indirect solar radiation, whereas the other five blade pieces were maintained in static conditions (without turnover). To simulate strong wave conditions in the open ocean, turnover of kelp tissue pieces was done every 10 min, a frequency that is considered conservative as real turnover time in natural conditions is most likely higher. Although the sunny and shadow sides are continuously changing in the turnover treatment, the identification of the initial blade position was registered to compare between the sides with the static treatment. The maximum quantum yield (Fᵥ/Fm) of the differently treated kelps was measured every 2 h between 09:00 AM and 7:00 PM, at 8:00 PM of the same day and then at 8:00 AM of the following day to evaluate recovery. On the basis of the daily variation of Fᵥ/Fm during the static cycle (first daily cycle), a sampling time of every 2 h was considered appropriate to evaluate the photosynthetic responses in turnover conditions.

To evaluate the effect of UVR on recovery of chlorophyll fluorescence (Fᵥ/Fm), a third daily cycle, referred to as ‘UVR cycle’, was conducted in full solar radiation (PAR + UVR) and without UVR (PAR) conditions. After exposing the kelp pieces for 1 h to full natural radiation (between 9:00 AM and 10:00 AM), initial values of Fᵥ/Fm were measured at 10:00 AM on both blade sides separately. Afterward, five randomly selected containers with blade pieces were kept under PAR conditions (Ultraphan 395-nm folder, 0.12 mm, Digesta, Munich, Germany), and the other five containers were maintained under PAR + UVR conditions (Ultraphan, 295-nm folder, 0.3 mm, Digesta). The maximum quantum yield was measured again at 2:00 PM, and then the containers were covered with a black mesh to induce recovery of photosynthesis by reducing the incoming solar radiation (see also Rothäusler et al. 2011a). The maximum quantum yield in each condition and on both blade sides was measured at 4:00 PM, 6:00 PM and 8:00 PM, respectively, and at 8:00 AM the next day. The blades were maintained static during the entire experimental time, without turning them over. The PAR and UVR attenuation beneath the black mesh was > 90% (portable quanometer MQ-200 and MU-200 respectively, Apogee Instruments, Logan, Utah, USA).

For the evaluation of the daily maximum quantum yield of PSII, two samples were taken from each blade piece with a cork borer of 13-mm diameter, and then the blade piece was put back in the experimental container in the original position. A transverse cut through the medullar tissue was done to separate the tissue section corresponding to the sunny and the shadow sides. Sample discs were maintained for 20 min in darkness before the measurements. The maximum quantum yield of chlorophyll a (Chl a) fluorescence (PSII) was measured in vivo with a portable fluorometer (PAM 2500 Waltz, Effeltrich, Germany). Measurements of Fᵥ/Fm were done randomly and each sample disc was represented by an average from two measures.

For each daily cycle, the effect of the different experimental conditions on photoinhibition (PhFᵥ/Fm) and recovery (RecFᵥ/Fm) were expressed as percent values in relation to the initial value LᵦFᵥ/Fm of maximum quantum yield. Photo-inhibition was calculated after Bruhn & Gerard (1996) using the equation:

\[ \text{PhF}_\text{v}/F\text{m} = 100 - \left[100 \times (L_\text{F}_\text{v}/F\text{m}/L_{\text{F}_\text{v}/F\text{m}})\right] \]

where LᵦFᵥ/Fm is the lowest value of maximum quantum yield found at noon. Recovery of PSII (RecFᵥ/Fm) from photo-inhibition was calculated with Fᵥ/Fm values at the next day with the following equation:

\[ \text{RecF}_\text{v}/F\text{m} = 100 \times (F_\text{F}_\text{v}/F\text{m}/L_{\text{F}_\text{v}/F\text{m}}) \]

where Fᵥ/Fm is the final value of maximum quantum yield at the end of recovery (8:00 AM the next day). Full recovery of PSII should be reached when final values are similar to initial levels as suggested by Roleda et al. (2006), and RecFᵥ/Fm approaches a value of 100.
In November 2011 and January 2012, tissue samples for soluble phlorotannin and pigment analyses were taken during the static cycle in a parallel set of experimental containers that were run specifically for this purpose. Ten independent blade pieces were used and the initial sampling was done at 8:00 AM, and then at 2:00 PM and 8:00 PM of the same day, and at 8:00 AM on the following day. Sunny and shadow samples were separated with a transverse cut in the medullar zone, frozen in liquid nitrogen, and later stored at −80°C. The analysis methods are the same used in previous studies with floating kelps (Roth et al. 2011a; Graiff et al. 2013; Tala et al. 2013).

The analysis of soluble phlorotannins was done with the Folin–Ciocalteu method as described in Gómez & Huovinen (2010) for ground dry samples. Three successive extractions were done for each sample (−15 mg dry weight (DW)) with 70% acetone for 12 h at 4°C. The absorbance was read at 730 nm in a multiscan spectrophotometer (Multiskan Spectrum 1500, Thermo Scientific, Vantaa, Finland) in a mixture of 100 μl of the extract, 100 μl of distilled water, 100 μl of Folin–Ciocalteu 1 N and 200 μl of 20% p/v NaCO3. The content of phlorotannins was expressed as mg g−1 DW and purified phloroglucinol (Sigma Aldrich, San Luis, Missouri, USA) was used as a standard.

For pigment extractions (Chl a, Chl c and total carotenoids) 15 mg of biomass [wet weight (WW)] was used for each sample, which was then incubated in the dark for 24 h at 4°C with 2 ml of N,N-dimethylformamide. Absorbances were quantified in a spectrophotometer (Spectro UV-2650, Labomed, Inc., Los Angeles, USA). Pigment content was calculated using the equations of Inskeep & Bloom (1985) for Chl a and those of Henley & Dunton (1995) for Chl c and total carotenoids. The values were expressed in mg g−1 WW.

The daily variation in \( F_v/F_m \) was analyzed for the static cycle experiment with a repeated-measures analysis of variance (ANOVA) for one between factor and two within factors. Month was considered as the between independent factor, because new kelps were collected bimonthly for each experiment (see above). Time of the day when seaweeds were measured was considered as a within factor as during the day the samples were taken from the same replicate. The two kelp sides were considered as the second within factor, because measurements of the sunny and shadow sides were taken from the same replicate. Ten replicates were considered for each combination of factors, leading to a balanced design.

In the three daily cycle experiments, the \( F_v/F_m \) recovery for the day after the photoinhibition was analysed using a linear mixed-effects model with R (R Core Team 2013) and lme4 (Bates et al. 2014). Month was considered a fixed effect because new kelp samples were collected bimonthly for the experiments (see above). Photoinhibition was also considered in the models because its effect was highly and inversely correlated with the recovery obtained in the experiments. In the first experiment (static cycle) month was considered as a main treatment; in the second experiment (turnover cycle) the effect of rotation (static or turnover) and in the third experiment (UVR cycle) the effect of solar irradiation (PAR or PAR + UVR) were included in the respective analysis. The side of the kelp (sunny or shadow) was considered as a random effect in the three analyses because of dependency of the two measures made on the kelp samples. Both the intercepts and the slopes for the effect of recovery were left to vary randomly across model estimations. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. P-values for the three experiments were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

In the experiment to evaluate phlorotannin and pigment changes during the static daily cycle in two seasons (November 2011–late spring, and January 2012–early summer), a total of five response variables was obtained from each side (sunny and shadow) of the same replicate. Considering the dependence (sunny and shadow sides sampled at different times of the day from the same replicate), a three-way nested model of a multivariate ANOVA (MANOVA) was considered most appropriate to determine significant effects (Quinn & Keough 2002). The main factor was month, the first nested factor was time of the day (four levels) and the second nested factor was side of the kelp (two levels). The analysis was done using SYSTAT v.13 (SYSTAT Software, Inc, Chicago, Illinois, USA).

**RESULTS**

**Environmental conditions**

During the course of experimentation, the environmental variables of radiation and temperature showed the expected seasonal variation. The daily radiation dose was lowest during the austral autumn/winter (March–September) and highest in summer (Fig. 1). The seasonal pattern was more notable for PAR and UV-A, with spring/summer values being almost twice as high as the fall/winter values (Fig. 1). Seawater temperatures also showed seasonal variations, with temperatures ranging from 12.8°C to 14.1°C in winter, and from 16.7°C to 19.3°C in summer (Fig. 1). The lowest daily temperatures were generally in the morning (8:00 AM) and the highest in the afternoon (3:00–5:00 PM).

**Daily cycles of photobiological performance**

The maximum quantum yield (\( F_v/F_m \)) during the static cycle showed a clear daily fluctuation with highest values in the morning and late afternoon, and lowest values in the early afternoon (Fig. 2). This pattern was observed during all months and for both blade sides in accordance to daytime PAR variations (Fig. 2). During the midday peak of solar radiation (between 12:00 and 4:00 PM), \( F_v/F_m \) was minimal, especially on the sun-exposed blade side. In September 2011 (early spring), when the lowest PAR levels occurred, the daily decrease in \( F_v/F_m \) was relatively minor compared with the other months (Fig. 2). The maximum quantum yields were especially low in November 2011 and January 2012, corresponding to late austral spring and early summer PAR, respectively (Fig. 2). Overall, \( F_v/F_m \) differed significantly for each tested factor (month, time, blade side), as well as for their interactions (Table 1).

The turnover and UVR cycles showed similar patterns in the daily variation of \( F_v/F_m \) (Figs S1, S2). Photosynthetic
efficiency was lowest on the sunny side and during spring/summer; this effect was more notable in the static than in the turnover treatment (Fig. S1). Moreover, in the turnover treatment, there were no clear differences in $F_v/F_m$ between blade sides. Similar values were detected between radiation treatments (PAR vs PAR+UVR), sides and months (Fig. S2).

As the blades in both experimental treatments were shaded after 2:00 PM, they started to recover during the afternoon, reaching similar or higher than initial $F_v/F_m$ values by the end of the day and maintaining these until the next day (Fig. S2).

Variations in the concentrations of metabolites (phlorotannins and pigments) were observed in November 2011 and January 2012 on both blade sides during the daily cycle. The three-way nested MANOVA showed that $F_v/F_m$ and phlorotannin concentrations differed significantly between months ($P < 0.001$), time of the day ($P < 0.05$) and for blade sides ($P < 0.05$) (Table 2; Table S1). In general, higher phlorotannin concentrations were found in January 2011 on the shadow side but the daily pattern depended on the month (Fig. 3). In November 2011, phlorotannin concentrations appeared not to change during the day, whereas in January 2012 a decrease in the concentration was observed toward the afternoon on both blade sides (Fig. 3).

**Photoinhibition and recovery of $F_v/F_m$**

The capacity of the floating kelps to recover their photosynthetic performance after photoinhibition partially depended on experimental conditions and photoinhibition levels (Figs 4, 5). In addition, photoinhibition was highly dependent on solar radiation, which varied between months according to seasonal

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**Table 1.** Results of repeated-measures ANOVA for the static daily cycle experiment to study the photosynthetic performance ($F_v/F_m$) of *Durvillaea antarctica*. Data were evaluated with a repeated-measures ANOVA between (months) and within subjects. The within subjects were time of the day and blade side (sunny and shadow) of each replicate.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>$F$-ratio</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subjects</td>
<td>18.6945</td>
<td>5</td>
<td>3.7389</td>
<td>155.9097</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>1.2950</td>
<td>54</td>
<td>0.0240</td>
<td></td>
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<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>20.0057</td>
<td>13</td>
<td>1.5389</td>
<td>689.6987</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Time × months</td>
<td>4.3377</td>
<td>65</td>
<td>0.0667</td>
<td>29.9085</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>1.5664</td>
<td>702</td>
<td>0.0022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side</td>
<td>9.0985</td>
<td>1</td>
<td>9.0985</td>
<td>1037.9326</td>
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</tr>
<tr>
<td>Side × months</td>
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<td>12.7035</td>
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<td>Time × side</td>
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<td>13</td>
<td>0.0823</td>
<td>38.7405</td>
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<tr>
<td>Error</td>
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<td>702</td>
<td>0.0021</td>
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**Table 2.** Summary of the three-way nested MANOVA test for the physiological response ($F_v/F_m$, phlorotannins) of *Durvillaea antarctica*. Main factor was month (November 2011 and January 2012), first nested factor was time of the day (8:00 AM, 2:00 PM, 8:00 PM and 8:00 AM recovery) and second nested factor was blade side of the kelp (sunny and shadow). Results of the univariate test are given for the response variables in which significant effects of the factors were identified. See text for details (full MANOVA is given in the online supplements, Table S1).

<table>
<thead>
<tr>
<th>Response variables, $P$</th>
<th>Month</th>
<th>Time (Month)</th>
<th>Side</th>
<th>[time (Month)]</th>
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</thead>
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<tr>
<td>Wilks’s lambda</td>
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<td>0.1149</td>
<td>0.1423</td>
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<tr>
<td>$F$-ratio</td>
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<td>5.7886</td>
<td>3.7285</td>
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<tr>
<td>df</td>
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<td>30, 242</td>
<td>40, 264</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
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<tr>
<td>Univariate $F$-tests</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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</tr>
<tr>
<td>Phlorotannins</td>
<td>&lt; 0.001</td>
<td>0.0176</td>
<td>0.0426</td>
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</tbody>
</table>
changes. The linear mixed models revealed no significant effects of month on the recovery of the $F_{v}/F_{m}$ values during the static cycle ($P = 0.0863$; Table 3). However, the interaction between month and photoinhibition in the static cycle was highly significant ($P = 0.0006$) for recovery capacity, reflecting the negative effect of higher solar radiation during summer. In the static cycle, no significant effect ($P > 0.05$) of blade side was detected on recovery capacity. Both blade sides showed

Fig. 2. Seasonal variation in the static daily cycle of maximum quantum yield ($F_{v}/F_{m}$) of *Durvillaea antarctica* blades in experimental tanks. Diurnal pattern of irradiance (PAR, μmol photons m$^{-2}$ s$^{-1}$) is shown as grey shading. Each point represents the mean ± standard deviation of 10 replicates for sunny (×) and shadow (Δ) sides.

Fig. 3. Phlorotannin and pigment concentrations in *Durvillaea antarctica* during late spring and early summer (November 2011 and January 2012) in the static daily cycle. Light grey columns represent the mean (± standard deviation) for the sunny side and dark grey columns represent the shadow side of the blade.
November 2011

Phlorotannins

mg g⁻¹ DW

Chlorophyll a

mg g⁻¹ WW

Chlorophyll c

mg g⁻¹ WW

Carotenoids

mg g⁻¹ WW

8 14 20 8

Day time Next day

Time (hours)

January 2012

mg g⁻¹ DW

mg g⁻¹ WW

mg g⁻¹ WW

mg g⁻¹ WW

0.0 0.1 0.2 0.3

0.0 0.1 0.2 0.3

Sunny Shadow
recovery in $F_v/F_m$ values after the night, but recovery tended to be less efficient on the sunny side during summer.

During the turnover cycle, higher photoinhibition and lower recovery capacities were observed on the sunny blade side, especially in the static treatment (Fig. 4). In this treatment, photoinhibition of the sunny blade side occurred in all months and was particularly evident during summer, where lower $F_v/F_m$ persisted until the next day (Fig. 4). Additionally, an increase in photoinhibition was also detected on the shadow side during summer (January 2011 and January 2012: 58% and 62%, respectively), but the recovery of $F_v/F_m$ was almost complete, reaching > 87% in both cases (Fig. 4, Fig. S1). In the turnover treatment, there were no clear differences in photoinhibition and recovery capacities between blade sides. Recovery of the experimental kelps was significantly ($P = 0.0059$) affected by the experimental treatment (static vs turnover; Table 3). Recovery capacity decreased by ~6% ($s_x = 2.100$, $P = 0.0059$) when experimental kelp pieces were maintained static compared with those that were continuously turned over. Furthermore, the recovery of the experimental kelp pieces was significantly affected by month ($P = 0.0264$) and also by the interaction with photoinhibition ($P < 0.0001$) (Table 3). Similar to the first static cycle, blade side did not significantly ($P > 0.05$) affect recovery, but in

Table 3. Linear mixed models for the effects of recovery ($F_v/F_m$) and photoinhibition of Durvillaea antarctica submitted to several effectors. The results for the three experiments are summarized. The random effects of blade side of the seaweed did not significantly ($P < 0.05$) affect recovery (see text for details).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>7</td>
<td>7</td>
<td>2.981</td>
<td>0.0863</td>
</tr>
<tr>
<td>Month × photoinhibition</td>
<td>8</td>
<td>131</td>
<td>3.722</td>
<td>0.0006</td>
</tr>
<tr>
<td>Turnover cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>7</td>
<td>7</td>
<td>4.888</td>
<td>0.0264</td>
</tr>
<tr>
<td>Static/turnover</td>
<td>1</td>
<td>134</td>
<td>7.825</td>
<td>0.0059</td>
</tr>
<tr>
<td>Month × photoinhibition</td>
<td>8</td>
<td>134</td>
<td>5.753</td>
<td>0.001</td>
</tr>
<tr>
<td>UVR cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>7</td>
<td>7</td>
<td>3.285</td>
<td>0.0696</td>
</tr>
<tr>
<td>PAR/PAR + UVR</td>
<td>1</td>
<td>134</td>
<td>4.879</td>
<td>0.0288</td>
</tr>
<tr>
<td>Month × photoinhibition</td>
<td>8</td>
<td>134</td>
<td>5.181</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
November 2011, January 2011 and January 2012 recovery of the sunny blade sides in the static treatment was very low (Fig. 4), probably as a result of the interactive effect between month and photoinhibition.

In the UVR cycle, solar radiation had a significant effect on the capacity of the kelp pieces to recover initial values of $$F_v/F_m$$ ($$P = 0.0288$$). Experimental kelps that were exposed to PAR only (without UVR) had recovery that averaged 5% ($$s_x = 2.071; P = 0.0288$$) higher than kelp pieces that were exposed to all components of solar irradiation (PAR + UVR) (Fig. 5). Recovery of experimental kelps maintained under the UVR treatment was not significantly affected by seasonality (month in our model) ($$P = 0.0696$$), but the interaction of month with photoinhibition was highly significant ($$P = 0.0001$$; Table 3). As in the other daily cycles, blade side did not affect recovery ($$P > 0.05$$; Table 3): sunny blade sides were able to recover in the same way as shadow sides (Fig. 5).

**DISCUSSION**

Daily acclimation in photosynthetic performance of *Durvillaea antarctica* depended on season, floating conditions and blade side. Increase of photoinhibition and a decrease in recovery capacity was more notable at high irradiance conditions, i.e. during summer and on the sunny blade side. Nevertheless, some floating conditions (e.g. continuous blade turnover, reduced daily dose of radiation) helped to mitigate the negative effects of solar radiation and temperature on floating kelps. Thus, intrinsic (photoacclimation potential, defense metabolites) and extrinsic factors (wave agitation of kelps, daily dose of radiation) determine the floating persistence of this bull kelp.

**Daily cycle of photosynthesis and metabolites**

Daily photosynthesis cycles for many plants, seagrasses and seaweeds generally show a decreasing activity around noon (Long et al. 1994; Silva & Santos 2003; Cabello-Pasini et al. 2000; Kokubu et al. 2015; Terada et al. 2016). We observed relatively strong diurnal changes in photosynthetic performance of *Durvillaea antarctica* relative to daily light cycles with maximum quantum yield, with low $$F_v/F_m$$ during early afternoon. This was consistent throughout the year for blade sides and experimental conditions. The decrease in the quantum yield of PSII is understood as a dynamic defensive mechanism in response to high irradiance. This avoids the photo-oxidative destruction of the photosynthetic apparatus and probably dissipates excess energy as heat (Häder & Figueroa 1997; Gómez et al. 2004; Gao & Xu 2010). Daily variations in maximum quantum yield were described for seaweeds in situ (Hanelt et al. 1993; Edwards & Kim 2010; Kokubu et al. 2015), as well as in outdoor (Cabello-Pasini et al. 2000; Cruces et al. 2013) and indoor culture tanks (Gómez et al. 2004; Abdala-Díaz et al. 2006; Terada et al. 2016). Accordingly, experimental conditions can help to understand the photosynthetic performance of *D. antarctica* during the first 24 h of floating.

Fig. 5. Seasonal variation of photoinhibition and recovery (%) of *Durvillaea antarctica* in relation to initial values of $$F_v/F_m$$ during the UVR daily cycle. In both case initial values were set at 100% (dashed line, see details in Material and Methods). Light grey columns represent the photoinhibition and dark grey columns represent the recovery. Each point represents the mean ± standard deviation of five replicates.
Phlorotannins, which are important secondary metabolites of brown seaweeds, have multiple functions due to their high antioxidant and photoprotective capacity (Swanson & Druelh 2002; Gómez & Huovinen 2010; Steinhoff et al. 2011). The adjustment of phlorotannin concentrations as a defensive response is a complex interaction between processes of exudation, photodegradation and synthesis. All of these can fluctuate on daily, seasonal and geographic scales (Van Alstyne et al. 1999; Connan et al. 2004, 2007; Abdala-Díaz et al. 2006, 2014). Herein, phlorotannins showed dynamic intraday changes, suggesting that variations are induced by photosynthetic stress due to an increase in solar radiation, as has also been reported for other brown seaweeds (e.g. Abdala-Díaz et al. 2006; Cruces et al. 2012, 2013). Lower phlorotannin values on the sunny blade side of Durvillaea antarctica could be caused by phenolic exudates and photodegradation. Laboratory experiments showed that phenolic compounds can photo-oxidize under visible light conditions (λ > 420 nm) (Kim & Choi 2005). The differences observed between spring and summer could be due to seasonal photoadaptive processes of acclimation in the field (Cabello-Pasini et al. 2000), as had also been observed in other seaweeds (Häder et al. 1996; Hanelt et al. 1997; Gómez & Huovinen 2010). Higher phenolic contents in summer were previously reported for other fucoids including Fucus vesiculosus Linnaeus, Himanthalia elongata (Linnaeus) S. F. Gray and Ascophyllum nodosum (Linnaeus) Le Jolis (Connan et al. 2004). The highly dynamic variation of phenol contents in D. antarctica reinforces the idea that the adjustments in phlorotannin concentrations serve as effective photoprotection strategy.

Pigments also present dynamic changes over time in response to variations in solar radiation (López-Figueroa 1992; Aguirre-von-Wobeser et al. 2000; Huovinen et al. 2006). However, the observed daily photosynthetic performance in floating Durvillaea antarctica did not appear to be related to variations in concentrations of chlorophylls and total carotenoids. Photoacclimation through changes in photosynthetic pigment generally occurs over timescales of days to weeks (Long et al. 1994; Ralph & Gademann 2005) or seasons (Gevaert et al. 2002). However, rapid dynamic changes in accessory carotenoids such as fucoxanthin, zeaxanthin, violaxanthin or antheraxanthin can occur over timescales of minutes or less (Ralph & Gademann 2005). Although total carotenoids did not mirror the photosynthetic diurnal changes, variation in their specific composition could be operating, especially those associated with the xanthophyll cycle (Vershinin & Kamnev 1996; Gevaert et al. 2002), an important protection mechanism under light stress.

**Photoinhibition and recovery of photosynthetic performance**

Photodamage depends on the intensity and duration of radiation exposure, and longer exposure requires more time for recovery from photoinhibition (Häder et al. 2001). Although photoinhibition is a mechanism of photodamage occurring throughout the year, a notable reduction in recovery capacity was observed during the summer, especially in Durvillaea antarctica blades floating in a static position. During the remainder of the year both blade sides fully recovered by the next morning, indicating the direct relationship between radiation dose and recovery potential. During *in situ* floating conditions, a decrease in the photosynthetic efficiency of *D. antarctica* was observed with increasing floating time (Graiff et al. 2013; Tala et al. 2016). Photoacclimation also occurred in benthic *D. antarctica* and a photoinhibition of 50% was reached in full solar radiation, which required at least 7 h to recover completely (Gómez & Huovinen 2011).

The particular morphology of *Durvillaea antarctica* with a highly buoyant, large blade generates specific conditions for floating. If the floating kelp is turned over continuously, e.g. by wave action, both sides of the blade are able to maintain a high recovery capacity. Irregular and fluctuating patterns of light can mitigate the negative effect of photoinhibition, allowing activation of photoprotective mechanisms that favour photosynthetic recovery (Dromgoole 1987; Greene & Gerard 1990; Wing & Patterson 1993; Roleda & Dethleff 2011). Recovery of photosynthesis after a photoinhibitory event decreases at high temperature (Hanelt et al. 1992; Bruhn & Gerard 1996; Cruces et al. 2013). This relationship reinforces the idea of negative effects during summer (combination of high solar radiation and temperature) on photosynthetic performance, compromising the persistence of floating seaweeds. In this sense, the recovery potential of floating kelps would depend on the environmental conditions that kelp rafts face at the sea surface (e.g. sunny vs cloudy days, winter vs summer, intense seawater movement and splashing vs calm sea conditions).

UVR has been described as an important factor that affects the physiological performance of seaweeds mostly on intertidal shores (Huovinen et al. 2006; Gao & Xu 2010). Although UVR had only minor effect on the daily cycle of $F_{i}/F_{m}$, the recovery capacity was enhanced in floating kelps that were only exposed to PAR. Relatively small effects of UVR were described in seaweeds that are confronted with high radiation levels in their natural environment, where PAR inhibition on sunny days can mask or add to the UVR effects (Hanelt & Roleda 2009). Long recovery periods may be necessary in UVR-induced photoinhibition (Hanelt et al. 1993), which can explain the difference in recovery levels between both radiation treatments. Although blade side itself had no effect on recovery in the UVR cycle, the high recovery capacity of the sunny blade side compared with the other daily cycles underlines the importance of daily radiation dose (which was shorter in the UVR cycle due to shading after 2:00 PM) in the photobiological response of floating kelps.

**Implications of environmental conditions for rafting dispersal potential**

In their benthic environment attached *Durvillaea antarctica* are moved by strong wave action, and blades are continuously turned over, causing an irregular light pattern on the thallus. In littoral habitats, some seaweeds can efficiently use light-pulses under fluctuating high light conditions with positive effects on overall productivity (Greene & Gerard 1990; Wing et al. 1993). When bull kelps are detached and float at the sea surface, they still move with the waves, but under moderate wave conditions they are no longer turned over, resulting in overexposure of one blade side to solar radiation. This effect is detrimental for the floating bull kelps, ultimately compromising the floating persistence, especially during the summer.
(Graiff et al. 2013). The shadow side of the blade receives mainly scattering and reflection radiation, resulting in higher levels of photosynthesis than observed on the sunny side of the blade. Possible translocation of organic compounds (Raven 2003; Gómez et al. 2007) between blade sides can occur to sustain sunny-side demands. Medullary filaments like sieve elements of Laminariales have been described in some Fucales (Roberts 1979; Moss 1983) as an anatomical system through which metabolite translocation can occur.

Along the coastal zone of central Chile, stronger winds occur in spring/summer, whereas in autumn/winter the winds are less intense, with a daily cycle of strong winds between 10:00 AM and 11:00 PM (Narváez et al. 2004). Thus, winds are strongest during the season and time of day with the highest solar radiation, facilitating the turnover of floating kelps and seawater splashing. These typical upwelling winds may not only cause floating kelps to tumble frequently, but also bring cooler waters to the sea surface (Aguirre et al. 2012), thereby possibly mitigating the negative impacts of solar radiation and high sea-surface temperatures during the summer and midday.

In conclusion, floating *Durvillaea antarctica* exhibit dynamic responses in photosynthetic performance and phlorotannin contents during the day. These are maintained across seasons with a high recovery capacity, regardless of the photoinhibition reached. Various factors can affect the persistence of floating seaweeds at the sea surface, such as the intensity of solar radiation (Huovinen et al. 2006; Graiff et al. 2013), sea-surface temperatures (Powles 1984; Rothhüsler et al. 2011a, b), grazing of organisms associated with kelp rafts (Cerda et al. 2009), wave movements and UVR (this study). In the study area (at the equatorward distribution limit of *D. antarctica*), under certain conditions the capacity to recover from photobiological stress appears to be compromised, resulting in photodamage, which ultimately can lead to reduced floating persistence and dispersal potential. Since kelps at the northern population range acclimated to conditions at the sea surface during most of the year, it is considered most likely that *D. antarctica* from central and southern Chile will survive the floating conditions even better, because the environmental conditions (temperature, solar radiation) are more moderate than at lower latitudes. This implies that *D. antarctica* has an overall high floating potential throughout its distribution range. However, examining seasonal variations in photoacclimation processes, growth and reproduction over the latitudinal range of *D. antarctica* could help to better estimate the floating persistence of this rafting kelp and evaluate the importance of rafting for population connectivity of *D. antarctica* and its associated biota.

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**SUPPLEMENTARY DATA**

Supplementary data associated with this article can be found online at [http://dx.doi.org/10.2216/16-93.1.s1](http://dx.doi.org/10.2216/16-93.1.s1).

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