Long-term persistence of the floating bull kelp *Durvillaea antarctica* from the South-East Pacific: Potential contribution to local and transoceanic connectivity

**Fadia Talaa,b,∗, Boris A. Lópezc, Marcel Velásquezd,h, Ricardo Jeldresa,f, Erasmo C. Macayae,f,g, Andrés Mansillaa,d,h, Jaime Ojedad,h,j, Martin Thiela,g,i**

a Departamento de Biología Marina, Universidad Católica del Norte, Larrondo, 1281, Coquimbo, Chile
b Departamento de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile
c Departamento de Acuicultura y Recursos Agroalimentarios, Universidad de Los Lagos, Avenida Pachulcocher, 1305, Osorno, Chile
d Laboratorio de Macroalgas Antárticas y Subantárticas (LMAS), Universidad de Magallanes, Facultad de Ciencias, Casilla 113-B, Punta Arenas, Chile
e Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile
f Centro FONDAP de Investigaciones en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Chile
g Millennium Nucleus Ecology and Sustainable Management of Oceanic Island (ESMOI), Coquimbo, Chile
h Instituto de Ecología y Biodiversidad, IEB-Chile, Universidad de Chile, Santiago, Chile
i Centro de Estudios Avanzados en Zonas Áridas, Coquimbo, Chile
j School of Environmental Studies, University of Victoria, Victoria, British Columbia, Canada

**ARTICLE INFO**

**Keywords:**
Chile  
*Durvillaea antarctica*  
Floating persistence  
Rafting  
Dispersal  
Floating seaweeds  
Temperature

**ABSTRACT**

Current knowledge about the performance of floating seaweeds as dispersal vectors comes mostly from mid latitudes (30°–40°), but phylogeographic studies suggest that long-distance dispersal (LDD) is more common at high latitudes (50°–60°). To test this hypothesis, long-term field experiments with floating southern bull kelp *Durvillaea antarctica* were conducted along a latitudinal gradient (30°S, 37°S and 54°S) in austral winter and summer. Floating time exceeded 200 d in winter at the high latitudes but in summer it dropped to 90 d, being still higher than at low latitudes (<45 d). Biomass variations were due to loss of buoyant fronds. Reproductive activity diminished during long floating times. Physiological changes included mainly a reduction in photosynthetic (Fv/Fm and pigments) rather than in defence variables (phlorotannins and antioxidant activity). The observed long floating persistence and long-term acclimation responses at 54°S support the hypothesis of LDD by kelp rafts at high latitudes.

1. Introduction

The connectivity between populations is an essential aspect for understanding population dynamics, dispersal and migration, gene flow, colonization of a new or altered habitat, and range expansion (Kool et al., 2013). In marine environments rafting on biotic and abiotic substrata is recognized as an important dispersal mechanism to connect close or distant populations. The significance of rafting as a transport mechanism is most relevant in species with limited autonomous dispersal capacities (i.e. absence of larvae or low mobility of propagules), with rafting allowing them to extend their distribution ranges according to their physiological/reproductive tolerances, ecological interactions and environmental factors (Thiel and Gutow, 2000; 2005a; McKenzie and Belgrove, 2008; Vandenriessche et al., 2007; Rothäusler et al., 2009, 2011a, 2011b; Graiff et al., 2016), even though phylogeographic studies have identified important transoceanic rafting routes at high latitudes (Fraser et al., 2016, 2018; Coyer et al., 2011; Rothäusler et al., 2012; Moon et al., 2017).

The subantarctic region is characterized by vast expanses of open ocean, with a few dispersed islands, the southern tip of South America...
There are no studies integrating biological and genetic aspects in which is highly indicative of LDD at high latitudes (Fraser et al., 2010). Islands and New Zealand (termed the Patagonia/subantarctic clade), has one single and widespread haplotype common to most subantarctic homogenous southern clade at >44°S in the Patagonian fjord region (Hay, 1994; Fraser et al., 2010; Cruces et al., 2012, 2013; Tala et al., 2016, 2017; Mansilla et al., 2017; Méndez et al., 2019). Short-term laboratory experiments have shown that an increase in 5°C has a significant impact on physiological variables in the kelps at 30°S, 36°S, and 53°S latitudes and that photodynamic acclimation was possible mainly in winter (unpublished data).

The SE Pacific temperate Chilean coast offers an excellent natural laboratory, characterized by a latitudinal gradient of sea surface temperature (Ramos-Rodríguez et al., 2012; Tapia et al., 2014) and solar radiation (Vernet et al., 2009). This spatial pattern in combination with seasonal changes in these variables (Huovinen et al., 2006; Rothäusler et al., 2009; Tala et al., 2016) produces an environmental variability that floating kelps must cope with during their rafting journeys. A longer floating time should be accompanied by an adequate physiological adjustment that allows maintaining biomass production and reproductive capacity when arriving in a new habitat. Therefore, D. antarctica (considering its positive buoyancy and wide geographic distribution throughout the subantarctic region) can be used as an ideal biological model to evaluate the performance of floating kelps under divergent conditions along an extensive latitudinal gradient.

Herein we tested the following hypotheses: (i) floating D. antarctica better adjust to sea surface conditions and therefore float for longer time periods at high than at mid-low latitudes, and (ii) winter environmental conditions are more beneficial to allow floating persistence than summer conditions. In order to test these hypotheses, in situ long-term experiments were conducted during contrasting seasons (winter vs. summer) at three sites along the SE Pacific coast (30°S–54°S) to determine changes in morphological (biomass change), physiological (chlorophyll a fluorcescents in PSII, pigments, pholorotannin contents, and antioxidant activity), and reproductive traits of floating bull kelp D. antarctica. These results will allow to understand the underlying mechanisms that facilitate the long persistence capacity of floating seaweed at high latitudes as suggested by phylogeographic studies.

2. Materials and methods

2.1. Experimental zones

Long-term field experiments were used to evaluate the spatial (latitude) and temporal (season) effects on floating capacity and performance of D. antarctica. The in situ experiments were started during austral winter 2014 (July) and late spring-summer 2014/2015 (December) at each locality. These experimental sets were henceforth named as winter and summer experiment, respectively. The experimental zones were at low (Coquimbo, Herradura Bay: 29°57′S, 71°20′W), mid (Concepción, Dichato: 36°29′S, 72°54′W), and high latitudes (Punta Arenas, Puerto del Hambre: 53°36′S, 70°55′W) (Fig. 1). These study sites (spanning a distance of more than 2,500 km) allowed examination of the spatial gradient in environmental conditions to which floating rafts are exposed along the SE Pacific coast, including extremes of low and high latitudes according to the distributional range of D. antarctica in Chile (López et al., 2018). Each experimental zone coincides with a different biogeographic region and districts based on Camus (2001) and López et al. (2018) (Septentrional District, from 30°S to 33°S; Mediterranean District, from 33°S to 37°S; and Magallanic Province, starting from ~46°S southward).

2.2. Sampling of benthic bull kelps for floating experiments

At each experimental site, individuals for the experiments were selected from the same benthic populations in both seasons. Entire thalli (n = 26–32 per season) of D. antarctica were detached with their...
holdfasts during low tide from benthic populations near the three experimental sites: Coquimbo (Puerto Oscuro: 31°25′S, 71°36′W), Concepción (Burca: 36°28′S, 72°54′W), and Punta Arenas (San Isidro Lighthouse: 53°47′S, 70°58′W) (Fig. 1). After collection, the experimental thalli were kept in a cooler at ambient temperature and immediately transferred to running seawater tanks in the laboratories where they were prepared for the experiments during the following day. Before the experiments, all individuals were thoroughly rinsed to remove epiphytes and fauna.

The thallus size of the collected individuals depended on the size structure of each sampled population, the weather conditions and the accessibility of each benthic population. Although the sizes of experimental thalli varied with latitude (sites) and season (Table S1), all collected individuals were adults with fully developed floating medulla. The individuals of *D. antarctica* differed significantly in total weight ($F_{2,118} = 7.433, P < 0.05$) and maximum length ($F_{2,179} = 13.162, P < 0.05$) only among sites (Table S2). Significantly ($P < 0.05$) higher weights were found for thalli from Coquimbo compared to Concepción and Pta. Arenas (Tables S1 and S2). All sites differed in thallus length, decreasing in size from Coquimbo to Pta. Arenas (Tables S1 and S2). Despite these differences, the size distributions showed extensive overlap among sampling sites and seasons, leading us to conclude that size/age had a negligible effect on the evaluated responses.

### 2.3. Experimental design of the long-term floating experiment

The experimental design did not consider reciprocal transplants because the experimental sites were situated in different biogeographic zones along the South-East Pacific (SEP) coast (Camus, 2001; Thiel et al., 2007; Försterra, 2009), where important variations in environmental conditions (such as e.g. temperature, solar radiation, and freshwater runoff) prevail, which cause important changes in species composition and genetic structure of seaweed clades with similar morphologies (reviewed in Guillemin et al., 2016). Similar differences between these biogeographic regions have been reported for many marine invertebrate taxa (e.g. Véliz et al., 2003; Varela and Haye, 2012; Haye et al., 2014; Trovant et al., 2015). Thus, artificial transport of *D. antarctica* and their epibionts across these biogeographic boundaries could potentially produce displacement of non-indigenous species generating undesired impacts on the local communities. Furthermore, logistic challenges in transporting large thalli could potentially affect their tissue and physiological integrity. For all these reasons no kelps were transplanted between sites. However, the responses of benthic and floating *D. antarctica* to different environmental conditions in winter vs. summer can be used to better interpret their acclimation and floating persistence within each biogeographic zone.

The same experimental set-up was used at the three experimental sites to test whether the independent variables latitude and season and their interaction cause variation in morphological/physiological performance of floating bull kelps and thus compromise their floating time. The experimental bull kelps were tethered (n = 18 to 29; see details in Tables S1b) to a long-line system with buoys (with 1–3 m distance) at the sea surface. Each bull kelp was identified with a tag on its main stipe and tied with a plastic cord to the long-line, allowing them to freely float at the surface, similar as described in Graiff et al. (2013) and Tala et al. (2016). Each tethered thallus was checked weekly to...
determine its day of sinking, and was considered as sunken when it was completely submerged. The time of sinking (expressed in days) was indicative of the floating persistence. Sunken thalli were removed from the long-line system and transported to the lab in order to take tissue samples and measurements (see details in point 2.5).

2.4. Environmental conditions during the study

Seawater temperature was monitored every 5 min using a HOBO® Pendant Temperature-Light Datalogger (Onset Computer Corporation, Bourne, MA, USA) throughout the experimental period at each experimental site. The dataloggers were attached 0.5 m below the sea surface in a horizontal position on each experimental line. Using all records from one day, the average daily water temperature was calculated for each experimental zone and during the experiment were obtained by satellite data from MODIS Aqua level 3, which are freely available from the NASA website (https://oceancolor.gsfc.nasa.gov/cgi/t3).

Additionally, the concentrations of seawater nutrients (mg L⁻¹) were determined at the beginning of each experiment. Seawater samples (n = 5) were collected at each site to measure concentrations of nitrate (NO₃⁻), nitrite (NO₂⁻), ammonium (NH₄⁺), and phosphate (PO₄³⁻) by spectrophotometric methods as described in Strickland and Parsons (1972). Samples were taken in 500 mL plastic flasks, fixed with approximately 3 drops of chloroform, and then frozen until analysis.

2.5. Sampling of benthic and floating bull kelps

Morphological, reproductive and physiological performance of initial (benthic status) and sunken bull kelps were measured using the same protocol as in previous studies with Macrocystis pyrifera (Rothäusler et al., 2009, 2018, 2011b; Graiff et al., 2016) and Durvillaea antarctica (Graiff et al., 2013; Tala et al., 2013, 2016). The initial performance status was determined for eight of the benthic thalli at the moment of collection (day 0). The thalli were randomly chosen to represent the experimental “population” from each site and season. The tethered individuals were then sampled at their sinking day to determine their performance changes using the same procedures at the three experimental sites (see details below). When a sunken thallus was detected, it was removed from the long-line systems and transported in a cooler to the respective nearby laboratory for further processing.

Tissue samples for the physiological variables (see details below) from benthic and sunken thalli were taken from the middle of the largest blade with a cork borer and gently cleaned from epibionts with soft paper towels and seawater as described in Tala et al. (2016). The tissue samples were obtained from photosynthetically active portions in order to evaluate the physiological changes at the time of sinking. Considering the large size of experimental thalli, a small piece of photosynthetically active tissue was suitable to determine physiological responses to environmental change as had been shown by previous studies of large seaweeds (e.g. Bischof et al., 1998; Connan et al., 2004; Rothäusler et al., 2011b; Gómez and Huovinen, 2011; Cruces et al., 2012). Blade samples for in vivo chlorophyll a fluorescence of photosystem II (PSII) were processed immediately in the laboratory. Samples for pigments, soluble phlorotannins and antioxidant activity were frozen in liquid nitrogen, stored at ~ 80°C. Samples from Concepción and Pta. Arenas were then sent on dry ice to the central analysis laboratory at Universidad Católica del Norte in Coquimbo.

2.6. Response variables of benthic and floating bull kelps

2.6.1. Morphological and reproductive changes

The total wet weight and maximum length of all experimental bull kelps were measured at day 0 (initial benthic status) and on the respective days of sinking. The size change expressed as % per day was calculated from the equation 
\[ \text{Size change} = \frac{(F_{t} - L_{0})}{L_{0}} \times 100 \] 
where \( F_{t} \) and \( L_{0} \) are the final (sinking time) and initial (day 0) size, respectively, in wet weight (BC) or maximum length (LC) of the experimental thallus at the respective sinking day (T).

The total (total wet weight and maximum length) of each experimental floating thallus was also measured every two weeks to determine the growth or tissue loss during the floating time. For this, each thallus was carefully removed from the experimental line and measured after which they were returned to their original position on the line. Biomass or length change (% d⁻¹) was determined as was described above.

A destructive sampling was carried out to determine the within-individual biomass distribution of D. antarctica for the initial benthic thalli (day 0; n = 8) and for the sunken thalli. The thalli were dissected into holdfast, stipes and frond, and the wet weight (g) of each portion was determined. Tissue weight for each portion was standardized with respect to the average value obtained from the initial benthic thalli for each portion (day 0) and the floating time of each one, thus expressed as relative change in biomass (% d⁻¹) for each tissue type.

The percentage of mature individuals was calculated considering the development stage of the conceptacles of each individual thallus for benthic (initial status) and sunken samples. Thin sections of the blade tissue were made and the maturity stages of a total of 30 conceptacles were examined for each individual (following the description in Lizée-Prynne et al., 2016). The mature stage was determined based on the conceptacle characteristics as fully developed gametes, sex differentiation and open ostioles. In this stage it is assumed that mature thalli are reproductively competent contributing with viable gametes to disperse and settle in a new habitat after rafting journeys. The results were expressed as percentage of mature individuals with respect to the total individuals evaluated.

2.6.2. Physiological performance

In order to evaluate the changes in the physiological variables during floating time, chlorophyll a fluorescence of PSII, concentration of chlorophyll a and soluble phlorotannins, and antioxidant activity were determined for the benthic (n = 8) and the experimental bull kelps when they had sunken from the sea surface. All physiological procedures were done in similar ways as in previous studies with D. antarctica (Graiff et al., 2013; Tala et al., 2013, 2016; Lizée-Prynne et al., 2016, 2017) and are briefly described below.

The maximal quantum yield (Fₚ/Fₐ) was considered as an indicator of photosynthetic activity and overall viability of the thallus, which is affected by stress conditions during floating time. To determine the maximal quantum yield of fluorescence (Fₚ/Fₐ), three small blade sections (~3 cm) from each thallus were cut off and incubated for 20 min in the dark and measured six times (two times each sections). Mean values of the six measurements represented the average response for each experimental thallus. The Fₚ/Fₐ was measured in vivo using a computer-aided portable pulse amplitude modulated fluorometer. However, due to logistic limitations and simultaneous experiments, different equipments were used at each of the three sampling sites throughout the experiments (Coquimbo: PAM 2500, Walz, Effeltrich, Germany; Concepcion: MINI-PAM, Walz, Effeltrich, Germany; Punta Arenas: DIVING-PAM, Walz, Effeltrich, Germany). Therefore, to visualize how photosynthetic conditions changed at sinking time and considering that different initial benthic values were observed among sites, final Fₚ/Fₐ values were expressed relative to the respective initial values (day 0). Considering the different technical characteristics of the equipments used (Figueroa et al., 2013), this transformation allowed better comparability between experimental sites and seasons.

From each benthic and sunken experimental thallus, a pool of three small blade disks was used for pigment extraction with N,N-di- methylformamide (DMF) for 24 h at 4°C in darkness. The Chl a content (mg g⁻¹ ww, wet weight) was calculated using the dichromatic
were mixed with 1250 mL of distilled water, 500 μL of Folin-Ciocalteu, and acetone, stirred for 24 h at 4°C. Subsequently, separation of the supernatant was performed by centrifugation (BOECO, U-320R, Germany) at 4500 rpm for 15 min and from the supernatant fraction 250 μL was performed by centrifugation (BOECO, U-320R, Germany). The procedure was repeated three times and the absorbance was measured at 730 nm in a UV–visible spectrophotometer (Rayleigh, model UV-1601, China). The values were calculated in mg chlorophyll g⁻¹ dw (dry weight) using Phloroglucinol (Sigma-Aldrich) as standard.

The radical scavenging activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams et al., 1995). The fresh tissue sample powder in nitrogen (~240 mg ww) was extracted with 1 mL 70% ethanol at 50°C for 60 min (homogenized every 15 min). 300 μL of the algal extract were mixed with 700 μL DPPH (50 mg L⁻¹ 70% methanol), and the decay of absorbance was measured at 0 and 30 min at 523 nm in an UV–visible spectrophotometer (Rayleigh, model UV-1601, China). The percentage of radical scavenging activity was calculated from the consumption of DPPH using formula [1−(Aₚ/A₀)] × 100, where Aₚ represents the absorbance of extract in solution after reaction with DPPH (at final time), and A₀ is the absorbance at time 0 (Molyneux, 2004).

2.7. Statistical analyses

To determine whether the sizes of experimental thalli differed between sites (Coquimbo, Concepción and Pta. Arenas) and seasons (winter and summer), the total wet weight and maximum length of the experimental D. antarctica were compared with two-way ANOVA. The weight and length data were log-transformed before being analysed to comply with requirements of normality and homoscedasticity. When the ANOVA revealed significant differences, a post-hoc Tukey’s HSD test was applied. Normality was verified using Shapiro–Wilks’s statistic and homoscedasticity of variance by using Levene tests (Underwood, 1997; Zar, 2010). Environmental conditions in each experimental zone were only analysed descriptively.

Floating persistence (days) curves by site and season were analysed using Kaplan-Meier survival analysis with a confidence level of 95%. The six Kaplan-Meier survival curves were compared with the Peto-Wilcoxon test. The survival analysis was done using the R software v3.4, “survival” package (R Development Core Team, 2018).

The total biomass and length changes per day (% d⁻¹) of sunken bull kelps were analysed with two-way ANOVA, separately for each variable (biomass, length). The factors site (Coquimbo, Concepción and Pta. Arenas) and season (winter and summer) were considered as fixed and a post-hoc Tukey’s HSD was applied when the ANOVA revealed significant differences. For the analyses, the constant 10 was added to each rate of daily percent biomass change to make them non-negative to allow log transformation (McDonald, 2014). The normality was verified using Shapiro–Wilks’s statistic and the homoscedasticity of variance by using Levene tests, respectively.

A permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was applied in order to examine whether experimental site (Coquimbo, Concepción and Pta. Arenas) and season (winter and summer) had an effect on the response variables of floating kelps at the time of sinking. The responses measured on the sunken kelps included tissue biomass changes with three variables (fronds, stipes, and holdfast biomass), and physiological performance, which included four variables (Fv/Fm, chlorophyll a, phlorotannins, and antioxidant activity). In all cases, the variables were standardized by the initial benthic tissue biomass or physiological status and the floating time (% relative to initial value d⁻¹) because benthic kelps differed according to site and season, and in floating time. The initial values were calculated from the mean of the eight benthic thalli (day 0) for each site and season. Two-way PERMANOVA tests were done considering site as random factor (three levels) and season as fixed factor (two levels). The similarity matrix for each experimental bull kelp was obtained through the Euclidean distance with 9999 permutations. Then post-hoc pair-wise comparisons were used to explore significant factor effects using 9999 permutations. After each PERMANOVA, a Similarity Percentage (SIMPER) routine was applied to verify the contribution of each biomass or physiological variable to the differences for each factor. This was done via a standardized (normalized) data matrix, using a 90-percentage cut-off, as well as the average dissimilarity between levels of each factor. The analyses were done using the statistical package PRIMER v7 (Clarke et al., 2014). When the PERMANOVA revealed significant effects of each factor, response variables were analysed individually with two-way ANOVA considering site (three levels), season (two levels), and their interactions (site x season) as factors. Normality of data and homoscedasticity of variance were verified using Kolmogorov-Smirnov statistics and Levene tests, respectively. Since data distribution within factors often violated normality assumptions and transformation failed, a conservative confidence level of 99% was used (Underwood, 1997). Significant differences between factors were evaluated using a Tukey HSD test (α = 0.01).

The frequency of the maturity stage of the initial benthic (n = 8) and experimental bull kelps at the time of sinking were analysed applying a log-linear model considering the factors status (benthic vs. sunken thallus), locality, season, and their interactions (Quinn and Keough, 2002). The Generalized linear model (GLM) with only the significant factors was glm (mature ∼ status + season + offset (log (total algae)), data = R2, family = poisson). All analyses were performed using the R software v3.4. (R Development Core Team, 2018).

3. Results

3.1. Environmental conditions during the study

Clear latitudinal and seasonal differences in local seawater temperatures and PAR were observed during the experiments. Lowest mean temperatures were registered in winter and at high latitudes and then increased towards the summer at all localities (Fig. 2). Higher variability in daily temperatures was detected during summer, especially in Concepción and at the end of the summer in Pta. Arenas. Monthly PAR showed a similar tendency as temperature, with high levels at low (Coquimbo) and mid latitudes (Concepción), especially during winter and spring month. During early summer, similar PAR levels were detected in the three experimental zones independent of latitude (Fig. 2).

During the experiments, the essential nutrients (N and P) were present in the experimental areas but concentrations were variable and depended on site, season and type of nutrient (Table S3). Higher concentrations of phosphate were detected in summer for the three experimental sites, while lower concentrations of nitrate were measured in Pta. Arenas in both seasons (Table S3).

3.2. Floating persistence of bull kelps

The survival of floating kelps significantly differed (P < 0.05) among sites, seasons and all pairwise combinations (Table 1). Rapid sinking was observed at the low latitude site (Coquimbo) with the first sunken thallus before 20 days. For thalli set afloat in the summer experiment, the maximum floating time was short and almost half of the experimental bull kelps had sunk after 30 days at both low and mid
latitude sites. Maximum floating persistence of *D. antarctica* was observed at the high latitude site (Pta. Arenas) where the experimental kelps floated for up to 203 days in winter and 90 days in summer experiments (Table S1b). During the last experimental summer floating bull-kelps in Concepción and Pta. Arenas sank after days with high seawater temperature (Fig. 2).

### 3.3. Performance of biomass, reproductive and physiological characteristics

The analysis of PERMANOVA showed significant differences (*P* < 0.01) among sites and the interaction site x season (Table 2). Floating kelps from Coquimbo and Concepción were significantly different from those in Pta. Arenas, and the holdfast biomass was the tissue that mostly contributed to these differences (Table 2). There were no significant differences between seasons. However, the significant interaction (*P* < 0.01) showed that the differences occurred in winter experiments between Coquimbo and Concepción and between Coquimbo and Pta. Arenas, and in summer experiments only between Concepción and Pta. Arenas (Table 2). The

### Table 1

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>X²</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WINTER (overall)</td>
<td>28.9</td>
<td>2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cq x Co</td>
<td>18.6</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Co x Pa</td>
<td>13.3</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SUMMER (overall)</td>
<td>65.3</td>
<td>2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cq x Co</td>
<td>15.4</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Co x Pa</td>
<td>44.2</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pta. Arenas W x S</td>
<td>41.2</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The analysis of PERMANOVA showed significant differences (*P* < 0.01) among sites and the interaction site x season (Table 2). Floating kelps from Coquimbo and Concepción were significantly different from those in Pta. Arenas, and the holdfast biomass was the tissue that mostly contributed to these differences (Table 2). There were no significant differences between seasons. However, the significant interaction (*P* < 0.01) showed that the differences occurred in winter experiments between Coquimbo and Concepción and between Coquimbo and Pta. Arenas, and in summer experiments only between Concepción and Pta. Arenas (Table 2). The
SIMPER analysis revealed that different variables contributed to the dissimilarity between sites, depending on season (Table 2). Changes in the physiological characteristics at the time of sinking showed a low contribution to the differences among the factors.

### 3.3.1. Biomass and length changes

At the time of sinking thalli had lost biomass and became shorter at all sites and in all seasons (Fig. 3). The biomass change presented significant effects ($P < 0.01$) for the factors site and season, while for daily length change significant effects ($P < 0.01$) were found for site and for the interaction site x season (Table 3). Daily biomass loss in Coquimbo was significantly ($P < 0.01$) higher than in Concepción and Pta. Arenas (Table 3, Fig. 3). In summer experiments, both low (Coquimbo) and mid (Concepción) latitude sites showed high loss in thallus weight and length (Fig. 3). Changes in thallus length depended on site and season, with significantly ($P < 0.01$) higher losses at mid latitudes (Concepción) than at the other sites (Table 3). The losses in weight and length during floating conditions were also observed during the bi-weekly control of *D. antarctica* (Figs. S1 and S2). Only the floating bull kelps that were set afloat in winter at Pta. Arenas showed an increase in the total length during the first three months of the experiment accompanied by minor changes in biomass (Figs. S1 and S2).

The within-individual biomass distribution showed that the sunken thalli lost mostly frond and stipe tissues, whereas the holdfasts contributed positively to the changes in total biomass (Fig. 4). In the initial status (benthic thalli at day 0), frond tissues represented more than 60% of the total biomass, but in sunken thalli this proportion decreased to ∼50%. Frond tissues showed an important reduction in biomass especially for thalli set afloat at low latitudes (Coquimbo) in winter and at mid latitude (Concepción) in summer experiments (Fig. 4). Minor changes were observed in frond biomass in Pta. Arenas. Only the biomass changes in fronds were significantly ($P < 0.01$) affected by the interaction site x season (Table S4). No differences in daily biomass changes of stipes and holdfasts were detected between sites and seasons (Table S4, Fig. 4).

### 3.3.2. Reproductive maturity

Benthic *D. antarctica* (initial status) showed a clear seasonal reproductive pattern with higher frequency of mature individuals during winter than in summer especially in Coquimbo and Concepción (Fig. 5).

---

**Table 2**

Results of two-way PERMANOVA for response variables in sunken Durvillaea antarctica. Factors were experimental sites (Cq = Coquimbo; Co = Concepción; Pa = Pta. Arenas) and season (W = winter and S = summer). Pair-wise post-hoc comparisons were done on significant terms. Significant values ($P < 0.01$) are shown in bold. Pair-wise tests show site, season or site x season interaction that differed ($P < 0.01$). Non-significant pair-wise tests are not shown. The response variables included daily biomass change of frond, stipes and holdfast, and physiological performance of Fv/Fm, chlorophyll $a$, phlorotannins and antioxidant activity. The results of SIMPER analyses were done for each significant pairwise comparison and are indicated for the variable with the highest % dissimilarity contribution.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>Pseudo F</th>
<th>P-value</th>
<th>Significant pair-wise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>2</td>
<td>83.116</td>
<td>4.8347</td>
<td>0.0002</td>
<td>Cq ≠ Pa; Co ≠ Pa</td>
</tr>
<tr>
<td>Season B</td>
<td>1</td>
<td>52.925</td>
<td>0.7482</td>
<td>0.5579</td>
<td></td>
</tr>
<tr>
<td>A x B</td>
<td>2</td>
<td>71.386</td>
<td>4.1524</td>
<td>0.0007</td>
<td>W [Cq ≠ Pa; Cq ≠ Co]S [Co ≠ Pa]</td>
</tr>
<tr>
<td>Residuals</td>
<td>107</td>
<td>17.191</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pairwise comparisons SIMPER**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dissimilarity Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holdfast (% d$^{-1}$)</td>
<td>37.30</td>
</tr>
<tr>
<td>Holdfast (% d$^{-1}$)</td>
<td>31.50</td>
</tr>
<tr>
<td>Phlorotannins (% d$^{-1}$)</td>
<td>35.11</td>
</tr>
<tr>
<td>Frond (% d$^{-1}$)</td>
<td>34.33</td>
</tr>
<tr>
<td>Holdfast (% d$^{-1}$)</td>
<td>43.05</td>
</tr>
</tbody>
</table>

---

**Fig. 3.** Daily total biomass and length change (% d$^{-1}$) of floating *Durvillaea antarctica* individuals (mean ± SD) from the start of the experiment to the day of sinking for thalli set afloat in winter 2014 and summer 2014/2015.
significant effects ($P < 0.01$) on the frequency of mature thalli of *D. antarctica* (Pseudo-$R^2 = 0.988$; Table S5). The proportion of mature individuals was substantially lower among sunken thalli than among benthic thalli at all sites (Fig. 5). In summer experiments, mature sunken individuals were only observed in Coquimbo (low latitude) and Pta. Arenas (high latitude) (Fig. 5).

### 3.3.3. Physiological performance

Negative changes in the relative maximum quantum yield ($F_{v}/F_{m}$) and chlorophyll $a$ concentrations were observed in sunken *D. antarctica* in both seasonal experiments and at all sites (Fig. 6A–B). The two-way ANOVA detected only significant ($P < 0.01$) differences for the interaction site x season in chlorophyll $a$ (Table S4). Summer conditions in Coquimbo and Concepción resulted in low chlorophyll $a$ levels in sunken thalli compared to their initial benthic status (Fig. 6B).

In contrast to the photosynthetic characteristics, phlorotannin concentrations increased in sunken thalli, mainly in Coquimbo (winter) and Concepción (both seasons) with positive values (Fig. 6C). Minor changes were observed in Coquimbo (summer experiment) and both seasonal experiments in Pta. Arenas. Variations in antioxidant activity were minimal compared to the other studied variables (mean < 0.15% d$^{-1}$). Positive changes indicating increases in antioxidant activity were observed in sunken thalli in Coquimbo (winter experiment) and in Pta. Arenas (summer experiment) (Fig. 6D). Both phlorotannin concentrations and antioxidant activity showed significant ($P < 0.01$) differences only for the interaction between site x season (Table S4).

### 4. Discussion

Floating persistence and physiological acclimation of *Durvillaea antarctica* depended on latitude and season. At high latitudes this kelp floated for more than 200 days while at low latitudes it survived for less than 50 days at the sea surface. Summer conditions, accompanied by an increase in temperature and solar radiation, led to relatively shorter floating times at low and mid latitudes (20% shorter than in winter), which was even more pronounced at high latitudes (55% shorter floating times in summer than in winter). The proportion of thalli with mature conceptacles were reduced during long floating times, suppressing the colonization potential of kelp rafts. The relative increase in holdfast biomass compared to the buoyant frond biomass appears to be more important for sinking of floating *D. antarctica* than physiological changes.

#### 4.1. Seasonal effect on floating persistence of bull kelps

Solar radiation and temperature are the main factors that provoke seasonal changes in biochemical and physiological traits impacting seaweed reproduction and growth (Kain, 1989; Falkowski and La Roche, 1991; Hurd et al., 2014). Seasonal changes in solar radiation are dependent on latitude showing a difference of 50% between winter and summer conditions at low latitudes, whereas at high latitudes radiation in summer can be 80% higher than in winter (Kain, 1989; Huovinen...
In summer conditions, the increase in solar radiation is accompanied by an extension of the light hours, and consequently the daily dose radiation can be higher at high than at low latitudes. These latitudinal differences in the summer-winter contrast in solar radiation can explain the steeper drop in floating persistence (∼50%) for *D. antarctica* set afloat in summer at high latitudes compared to low and mid latitudes (∼20%). Nevertheless, the experimental rafts of *D. antarctica* floated for substantially longer times at high latitudes than at mid or low latitudes during both seasonal experiments.

Negative biomass and length changes during floating time indicate a continuous loss of tissue, especially in summer experiments. The loss of frond tissue, fundamental for buoyancy, mainly contributed to these negative changes. Similar results have been described in floating *Macrocystis pyrifera* with rapid degradation and sinking under unfavorable environmental conditions, which can be accelerated by herbivory and epibiont loads (Rothäusler et al., 2009, 2018; Graiff et al., 2016). We did not measure the epibiont load in our experiments, because an overall weak epibiont cover was observed on the surface of fronds and holdfasts. Possibly, the continuous loss of the first cell layers of the fronds during prolonged floating times, prevented the establishment of epibionts.

Interestingly, the bi-weekly biomass control of floating bull kelps during the experiments allowed to detect increases in thallus length during the first three months in thalli set afloat in winter at high latitudes. During short-term floating time experiments (< 14d), *D. antarctica* was able to show positive growth in winter at high latitude despite the low light and temperature conditions (Tala et al., 2016). Lower or moderate temperatures can buffer negative effects of high solar radiation occurring in summer, whereas a deleterious combination of high temperature and light can affect the tissue integrity and accelerate biomass losses, thereby compromising the floating capacity of buoyant kelps (Rothäusler et al., 2009; Graiff et al., 2013; Tala et al., 2016). Lowest growth rates have been reported under high light intensities and high temperatures in *Ecklonia radiata*, which contrasted with highest growth rates at low temperatures (Bearham et al., 2013). The negative effect of high temperature and solar radiation on photosynthesis and activation of photoprotective mechanisms (Cruces et al., 2012, 2013) can be reflected in low biomass production.

The morphological changes during floating were accompanied by minor changes in physiological traits such as maximum quantum yield, pigments and phlorotannins. The photobiological characteristics of *D. antarctica* (i.e. low saturation irradiance, high efficiency photosynthesis, and dynamic photoinhibition) are considered adaptations for efficient light use (Gómez and Huovinen, 2011; Cruces et al., 2013), which might be associated with the subantarctic origin of this species. A notorious decrease in photosynthetic performance (*Fv/Fm* and pigments) was observed at the time of sinking especially in summer experiments at both low and mid latitudes. Similar changes in physiological responses of floating *D. antarctica* had been described in previous long- (Graiff et al., 2013) and short-term experiments (Tala et al., 2016), as well as in *M. pyrifera* (Rothäusler et al., 2011b). During

---

**Fig. 5.** Percentage of mature individuals of *D. antarctica* at day 0 (benthic) and at day of sinking for thalli set afloat during winter 2014 and summer 2014/15 experiments. Upper number represents the total number (mature and immature) of thalli controlled in each case.

---

**Fig. 6.** Daily change (% d$^{-1}$) of relative maximal quantum yields (A), chlorophyll a concentration (B), Phlorotannin concentration (C), and antioxidant activity (D) during the floating time of *Durvillaea antarctica* for thalli set afloat in winter 2014 and in summer 2014/2015. Values were standardized with respect to the initial mean value of benthic thalli and floating time.
floating, the physiological acclimation of *D. antarctica* might be insufficient to support growth and extend the persistence time, especially in summer, when the accelerated tissue degradation of the buoyant frond causes sinking of the kelp due to the heavy holdfast.

Seasonal metabolic performance and morphological changes during floating can also affect the reproductive potential of floating seaweeds. An important trait to consider after extensive floating journeys and in species with short-range propagules (Santelices, 1990) is the ability to remain reproductively competent upon arrival in new habitats. *Durvillaea antarctica* shows a strong seasonal reproductive pattern in benthic (Santelices et al., 1980; Hay, 1994; Collantes et al., 1997, 2002; Mansilla et al., 2017) as well as in floating conditions (Tala et al., 2013, 2016; Lizée-Pryne et al., 2016). Depending on latitude, gamete development can begin in autumn and extend to early spring whereas in summer the senescent stages predominate. As low sea surface temperatures favour the maturation and release of gametes (Hay, 1994) more than one reproductive event could occur during prolonged floating times at high latitudes. However, a drastic reduction in the proportion of mature thalli observed at the moment of sinking limits the availability of gametes for dispersal. When mature benthic bull kelps are detached in winter (i.e. high reproductive frequency) and rafting journeys take more than 3 months, the conceptacles degrade from mature to senescent stages losing their reproductive potential. Thus, rafting may suppress the availability of gametes, further reducing the potential of successful fertilization between floating and resident bull kelps.

### 4.2. Latitudinal effect on floating persistence of bull kelps

The persistence and performance of floating *D. antarctica* depended on the latitudinal conditions, and in almost all studied variables their responses were determined by the interaction between latitude and season. Environmental conditions at high latitudes favour an extended floating persistence, which decrease towards low latitudes. However, latitudinal differences in solar radiation and temperature are more pronounced in winter than in summer affecting physiology and growth patterns in seaweeds (Kain, 1989). During summer, a sudden increase in seawater surface temperature coincided with fast sinking of the last floating experimental thalli at high and mid latitudes (see Fig. 2) showing the importance of seawater temperature over floating persistence and tissue integrity.

The capacity of floating kelps to persist for long time periods at high latitudes is concordant with the common abundance of floating kelps in the subantarctic and cold regions of the southern hemisphere (Smith, 2002; Hinojosa et al., 2011; Wichmann et al., 2012; Fraser et al., 2017). At low and mid latitudes, the floating persistence of *D. antarctica* is limited mainly in summer as had been previously described by Graiff et al. (2013) and Tala et al. (2013), and also for the floating kelp *M. pyrifera* (Rothäusler et al., 2011b; Graiff et al., 2016). Although some specimens of floating *D. antarctica* have been found beyond the northern distribution limit (~30°S) of this species (Tala et al., 2013; López et al., 2017), only some of them showed long floating times based on sizes and frequency of stalked barnacles *Lepas* spp. The low floating persistence of *D. antarctica* can explain the low genetic connectivity of bull kelp populations in that area (Fraser et al., 2010).

At high latitudes sunken thalli showed minimum changes in photosynthetic traits and phlorotannins, and an increase in antioxidant capacity in summer experiments in contrast to low and mid latitudes. Also in short-term experiments (<14d), the floating kelps were able to maintain high antioxidant capacity and a latitudinal gradient was observed in biomass and physiological responses (Tala et al., 2016). Even though physiological acclimation might be occurring in each experimental zone, accelerated tissue disintegration, especially of photosynthetic tissue (fronds), causes enhanced fragmentation of the thallus, thereby limiting its floating time. In this sense, the loss of biomass could occur due to the breaking of larger frond pieces, some more deteriorated than others, which can float separately from the complete thallus for unknown times. Since these pieces (without a holdfast) exert a lower drag force against currents, they might travel faster and over longer distances compared to a complete, heavier thallus (with holdfast). Moreover due to their smaller sizes, the tissue pieces can be constantly turned over at the sea surface facilitating photosynthetic recovery and prolonged floating times (Tala et al., 2017). Large holdfasts, without floating capacity, would end up sinking thereby contributing to deep sea productivity (Krumhansl and Scheibling, 2012).

Although two divergent clades have been described for *D. antarctica* (Fraser et al., 2009, 2010), our results showed similar morphological changes associated with loss of biomass and length in floating conditions accompanied by slight variations in physiological traits such as maximum quantum yield, pigments and phlorotannins at all latitudes. The differences in biochemical composition and ecophysiological traits between the two clades of *D. antarctica* were relatively minor, much smaller than differences observed between two congener kelp species of *Lessonia* (*L. berteroana* Montagne and *L. spicata* (Suhr) Santelices, cryptic species of the Chilean coast) (Koch et al., 2015). In the case of *Durvillaea*, variations between thalli from Coquimbo and Concepción, corresponding to the same clade, were also observed in some morphological and physiological traits as part of the environmental local acclimation. Consequently, we infer that both clades have the ability to acclimate to environmental variables in very similar ways and thus the observed differences in floating persistence are due to differences in environmental conditions rather than to possible functional differences between the two genetic clades. In this sense, even though the subantarctic region provides favourable environmental conditions, the rapid decrease in floating persistence at high latitudes in summer, similar to what has been observed at low and mid latitudes reinforces the importance of environmental factors (i.e. temperature, solar radiation), affecting tissue integrity and floating persistence more than the biological/functional differences that could exist between the clades.

The records of *D. antarctica* pieces on Antarctic coasts, thousands of km from the nearest source populations (i.e. by molecular analysis) (Fraser et al., 2018) reinforce the importance of our study, identifying the physiological traits that facilitate the high floating capacity of bull kelps at high latitudes. The contribution of floating kelps to population connectivity of the kelp itself and of epibionts between distant coasts has been based only on molecular studies and more recently integrating ecological and geological assessments (Waters et al., 2018). Our results strongly support the hypothesis that the floating capacity of *D. antarctica* is substantially higher at high than low and mid latitudes.

### 4.3. Implications for long-distance dispersal of floating seaweeds

Abundant individual or group rafts of *D. antarctica* are widespread in the Southern Ocean, connecting adjacent and distant populations (Smith, 2002; Fraser et al., 2017, 2018). Herein environmental factors such as temperature and solar radiation (depending on latitude and/or season) were shown to affect persistence of kelps at the sea surface. Although the floating time persistence can be extensive, oceanographic and climatic factors such as strong surface winds and current velocity can enhance either dispersal or retention of floating kelps (Poulain et al., 2009; Waters and Craw, 2018). Therefore, how far the floating kelps can be dispersed depends on a combination of multiple factors that create an interaction between their acclimation potential and the environmental pressures at the sea surface. The Antarctic Circumpolar Current flows west-to-east around Antarctica with surface current speeds of 0.1–0.6 m s⁻¹ (Meredith et al., 2011). Depending on the speed and thallus size, the maximum floating time of 210 days found at high latitudes during winter can involve a travel between 1,000 (low speed) to 10,000 km (high speed). This estimate supports again the hypothesis that the high acclimation potential of *D. antarctica* at high
latitudes with more benign environmental (i.e. lower temperature) fav- ors long-term floating persistence and enhances the possibility of LDD.

The differential floating persistence observed in our study could also contribute to understand the spatio-temporal variability in the abundance of floating rafts (Hinojosa et al., 2010; López et al., 2017, 2019) and the heterogeneous genetic structure (Fraser et al., 2009, 2010) described for D. antarctica along the continental coast of Chile. Lower floating persistence at low and mid latitudes and especially in summer conditions restrict the potential for LDD, limiting connectivity between populations. At higher latitudes, the longer floating persistence can favour LDD and transoceanic dispersal with high abundance of floating thalli, thereby contributing to the homogenous genetic structure of bull kelp populations in the subantarctic region.

**Authors' contributions**

FT, AM, ECM and MT conceived the idea; FT, MV, RJ and JO collected the data; analyses were conducted by FT, MV, and BAL; FT, BAL and MT led the writing with assistance from MV, ECM, AM, RJ and JO.

**Conflicts of interest**

We have no competing interests.

**Funding**

The research was supported by FONDECYT (Chile) grant 1131023 to FT, MT, ECM and AM., CONICYT PAI (Chile) 79160069 to FT, and PhD-fellowship Beca CONICYT-PCHA/DoctoradoNacional/2014–21140010 to BL.

**Acknowledgements**

We gratefully thank Vieia Villalobos and Alvaro Villena, for their invaluable help and assistance in the field and laboratory procedures. Also we thank the undergraduate students from our universities in Coquimbo, Concepción and Pta. Arenas for their help in the different activities. We are also grateful to Dr. Carlos Lara (CIRENYS, Universidad Bernardo O’Higgins, Chile) to provide solar radiation information. Valuable comments by two anonymous reviewers also helped to improve the final version of the manuscript. Finally, we thank the support of Servicio Hidrográfico de la Armada de Chile (SHOA-CENDHOC).

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.05.013.

**References**


Fraser, C.I., 2017. Algol parasite Hetrodocia durvillaeae (Phaeophyceae): sphecalacites inferred to have traversed the oceanic Pacific with its buoyant host. J. Phycol. 49, 202–206.


Gutow, L., Giménez, L., Boos, K., Saborowski, R., 2009. Rapid changes in the epifaunal activities. We gratefully thank Vieia Villalobos and Alvaro Villena, for their invaluable help and assistance in the field and laboratory procedures. Also we thank the undergraduate students from our universities in Coquimbo, Concepción and Pta. Arenas for their help in the different activities. We are also grateful to Dr. Carlos Lara (CIRENYS, Universidad Bernardo O’Higgins, Chile) to provide solar radiation information. Valuable comments by two anonymous reviewers also helped to improve the final version of the manuscript. Finally, we thank the support of Servicio Hidrográfico de la Armada de Chile (SHOA-CENDHOC).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.05.013.

References


