Dominic Lizée-Prynne, Boris López, Fadia Tala and Martin Thiel*

No sex-related dispersal limitation in a dioecious, oceanic long-distance traveller: the bull kelp *Durvillaea antarctica*

Abstract: Dispersal of dioecious floating seaweeds could be limited due to biological constraints. This study examined for benthic and floating populations (stranded individuals) of the rafting kelp *Durvillaea antarctica* whether male and female individuals cohabit within one holdfast. As a previous study had indicated colour differences between sexes, we also examined whether these colour differences are consistent and possibly related to pigment and phlorotannin concentrations. Our large-scale survey of rafted holdfasts and a small-scale survey of benthic holdfasts at two sites found that reproductive males and females do travel together in coalesced holdfasts, although this proportion is relatively low (5–17%). There were no sex-specific differences in pigment and phlorotannin concentrations, but there were significant differences between the two benthic populations. There was no relationship between the colouration of thalli and the concentration of pigments but there was a slight colour difference between vegetative and reproductive sexual stages. Based on these results we conclude that biological conditions are not the cause for the lack of genetic connectivity between *D. antarctica* populations from central

Chile. Instead, we suggest that ecological processes, such as density-blocking and physical factors (i.e. currents and winds), limit the potential for successful rafting dispersal.

Keywords: chemical composition; dioecious seaweeds; dispersal potential; phlorotannins; pigments; rafting.

Introduction

For any successful dispersal to occur it is crucial that viable propagules reach new environments. In sexually reproducing species this implies the arrival of members of both sexes that can successfully reproduce and generate subsequent generations. Depending on the species, the probability of successful colonisation of new habitats can be considerably lower in sexually reproducing species than in asexually reproducing species, where any single individual can produce multiple propagules. This is especially true for species in which the sexes are separate due to their dioecious sexual system. Among these dioecious organisms are many brown seaweeds (Luthringer et al. 2014), some of which have been reported as long-distance dispersers based on genetic evidence (Fraser et al. 2010a, Coyer et al. 2011). Long-distance dispersal has been suggested for some positively buoyant dioecious seaweeds that are able to maintain viable propagules over long periods of time, as is the case for *Fucus vesiculosus* Linnaeus 1753 (Vandendriessche et al. 2007, Coyer et al. 2011) and *Hormosira banksii* (Turner) Decaisne 1842 (McKenzie and Bellgrove 2008).

Long-distance dispersal has allowed the spreading of many dioecious species, terrestrial or aquatic, which employ diverse strategies to ensure transport and establishment of viable propagules. For instance, certain seaweeds may be dispersed via the consumption of their spores by various herbivores and their release via the excrements of the animal or by having their spores stick to body appendages (Buschmann and Bravo 1990). In dioecious animals that have internal fertilisation, sperm storage may enhance the probability of simultaneous travel of both sexes (Shine et al. 2001, Friesen et al. 2014).

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Other dioecious species feature sexual parasitism, where the significantly smaller males attach themselves to the females for various reasons, such as nutrition, and release sperm whenever the dispersing female is ready to spawn (Vieira et al. 2013).

In dioecious seaweeds, each individual will either be male or female (in the haploid or diploid phase). Among the brown seaweeds, dioecy during the haploid phase is most prevalent (Luthringer et al. 2014) and dioecy during the diploid phase is exclusive to species of the order Fucales, which may or may not be buoyant. As certain of these dioecious species travel thousands of kilometres before reaching new potential colonisation sites, the probability of simultaneous arrival of both sexes is limited. However, some large brown seaweeds exhibit holdfast coalescence joining multiple conspecifics (González et al. 2015). If this coalescence unites individuals of both sexes in the same holdfast and both travelling sexes are sexually mature at the time of arrival, the probability of long-distance dispersal increases considerably.

Various environmental factors and biological traits can also limit the colonisation potential of long-distance travellers. For instance, a small number of propagules arriving from a distant population may have limited chances to establish themselves within a dense local population as a result of density-blocking (Waters et al. 2013, Neiva et al. 2014), when most available space is already occupied and propagules of new arrivals will be strongly outnumbered by local propagules. On the other hand, the biological traits of a species, e.g. thermal tolerance limits or tolerance to solar radiation at the sea surface, may reduce its potential for long-distance dispersal under certain conditions. For seaweeds it has been shown that warmer water temperatures or higher solar irradiance limit their reproductive potential (Macaya et al. 2005, Rothäusler et al. 2009). Furthermore, in dioecious seaweeds, one sex may be more susceptible to environmental factors than the other (Delph 1999), and thus lose its reproductive potential during long-distance voyages. For example, male individuals of the red seaweed *Asparagopsis armata* Harvey 1855 are more susceptible to herbivory than females because they do not invest as much energy in anti-herbivore defences (Vergés et al. 2008). Also, in dioecious plants, males and females can present substantial physiological differences (Cornelissen and Stiling 2005). This phenomenon of sexual differentiation also occurs in other seaweeds, such as the red seaweed *Gracilaria chilensis* Bird, McLachlan and Oliveira 1986 (Guillemin et al. 2014). However, this variability remains to be properly quantified for brown seaweeds (Thornber 2006). In summary, there may be a variety of ecological factors

and biological traits that limit the dispersal potential of dioecious seaweeds, even in species where both sexes can be united in the same holdfast.

To study the potential of long-distance dispersal in a dioecious species, the brown seaweed *Durvillaea antarctica* (Fucales) was selected as a model species. Molecular studies have confirmed that long-distance dispersal of this large kelp has occurred in the subantarctic region over distances of thousands of kilometres (Fraser et al. 2009, 2010b). However, for a distinct clade from the continental coast of Chile, there are extensive regions (200–300 km coastline) with unique haplotypes, and there appears to be very limited exchange between these regions (Fraser et al. 2010b). Thus, there are factors, ecological or biological, that suppress the dispersal potential of this particular clade of *D. antarctica* along the Chilean continental coast (Fraser et al. 2010b).

In Chile extensive benthic populations of *D. antarctica* occur between 30°S, its most northerly location (Cheshire et al. 1995), and 44°S, the southernmost confirmed location of the continental clade (Fraser et al. 2010b). Consequently, it could be that dispersal is suppressed due to the limited chances of successful immigration into dense populations. However, it is also possible that holdfasts rarely contain individuals of both sexes. Currently, no information is available about the sex and maturity of individuals that travel together with a coalesced holdfast. Furthermore, it is possible that one sex is more sensitive to environmental conditions than the other. In *D. antarctica*, males were reported to be of a yellowish colour whereas females have a darker, black-brownish colour (Collantes et al. 1997). This could indicate important chemical differences between the sexes that could impact their dispersal potential. Male and female individuals usually have differential energy allocation, where females invest a lot more energy into reproduction and protection of the gametes (Delph 1999, Cornelissen and Stiling 2005, Vergés et al. 2008). This variation has a direct impact on the chemical composition of each individual. For instance, one could expect that females might produce more phlorotannins, as this phenolic compound has repeatedly been proven to have a protective role, such as having high antioxidant activity in seaweeds (Li et al. 2009, Wang et al. 2009, Onofrejová et al. 2010). Additionally, the concentration of pigments, which are essential for photosynthesis and photoprotection, can vary between sexes (Guillemin et al. 2014).

The current study thus aims to (i) determine whether *D. antarctica* rafts comprise coalesced holdfasts with both male and female individuals, and (ii) evaluate if male or female individuals show different concentrations of various chemical components, reflecting poor resistance

to floating conditions and interfering with long-distance dispersal. We hypothesize that a significant number of holdfasts contain individuals of both sexes that also mature in synchrony, but that male and female individuals differ significantly in chemical composition of pigments and phlorotannins, resulting in sexual differences in dispersal potential.

Materials and methods

Study sites and sampling of benthic specimens

We studied the central Chilean clade of *Durvillaea antarctica* (Chamisso) Hariot 1892, which grows in dense benthic populations in the low intertidal zone of rocky shores between 30°S and 44°S (Cheshire et al. 1995, Fraser et al. 2010b). In order to obtain information on coalesced holdfasts, in particular the maturity stage and sex distribution of travelling individuals, recently stranded rafts with holdfasts were collected on 33 beaches during winters 2013, 2014 and 2015 as the fertile phase of *D. antarctica* is primarily found between austral autumn and spring (Santelices et al. 1980, Collantes et al. 2002) (Figure 1). A total of 1044 kelp rafts with holdfasts were evaluated for both their sex and their maturity stage (see below). For each intact organism with complete holdfast, the number of stipes per holdfast was determined. If a holdfast had more than five stipes (=individuals), only the five largest stipes were sampled. In the following we will use the term stipe to refer to an individual (consisting of its stipe and corresponding blade) within a holdfast. For a detailed examination of sex and biochemical composition, kelps were sampled from benthic habitats at two locations in northern-central Chile, Puerto Oscuro (31°25′22″S; 71°35′48″W) and Totoralillo Sur (32°05′07″S; 71°31′41″W). These samples were collected during June 2015 as the individuals would mostly be in their fertile phases (Figure 1).

We collected 56 benthic *D. antarctica* holdfasts in Puerto Oscuro and 62 holdfasts in Totoralillo Sur, in order to sample approximately 100 stipes, or individuals, per beach. Holdfasts were detached from rock surfaces in the lower intertidal zone using a hatchet, taking care to minimize mechanical damage of the individual. The entire organism was then weighed with the holdfast before cutting each individual at the base of its stipe. Each individual was then weighed on its own and measured from the base of the stipe to the tip of the blade. For this part
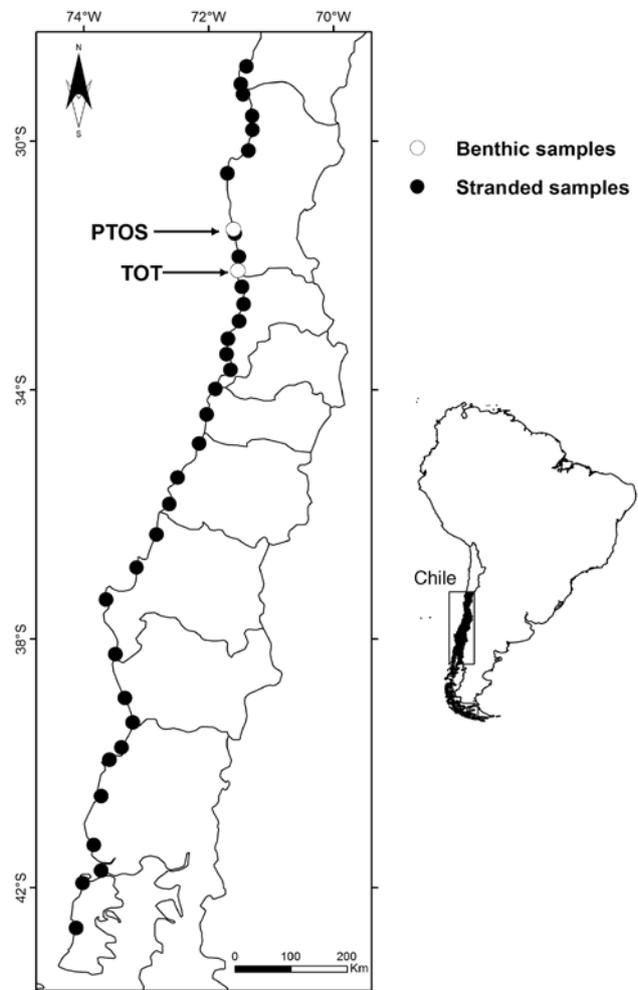

Figure 1: Study area and spatial distribution of the stranded and benthic *D. antarctica* in central Chile. PTOS, Puerto Oscuro; TOT, Totoralillo Sur.

of the study we only used individual holdfasts that had at least 250 g total weight (including holdfast, stipes and blades) and individual stipes that were at least 50 cm long, because at this size the individuals start to become sexually mature (Santelices et al. 1980, Collantes et al. 2002).

Three samples were taken from each individual stipe: (1) a large blade sample in order to determine the sex and maturity stage of the individual, (2) a small blade sample to determine the colour, and (3) a small blade sample for pigment and phlorotannin analyses. Of the approximately 100 stipes that were collected per beach, eight reproductive samples of each sex as well as eight vegetative samples were selected for determination of phlorotannin concentrations. The large blade samples were individually placed in large Ziploc® bags and covered with salt to dehydrate and preserve the reproductive tissues. The small pigment and phlorotannin samples were kept in

individual small bags, transported in darkness in a cooler, and frozen immediately upon return to the laboratory (within 12 h after field sampling) in order to maintain the pigments and phlorotannins intact.

Sex and maturity stage determination

The sex of *Durvillaea antarctica* can only be determined with full accuracy when gametes are identified by means of histological observation. Male conceptacles contain many small antheridia containing 64 sperm cells each, and female conceptacles contain multiple larger oogonia, with four large, dark oospheres within each oogonium. Using the large blade samples, thin transverse cuts were examined microscopically to determine both the sex and maturity stages of the conceptacles. As each stipe of a holdfast represents a single individual (González et al. 2015), the maturity of 30 conceptacles per stipe were determined from different cuts following Collantes et al. (2002) in order to describe the maturity stage of each stipe, which was done by identifying the most abundant conceptacle maturity stage. The current study aimed to determine whether the stipe was vegetative or reproductive, and thus we did not distinguish between the different maturity stages described by Collantes et al. (2002). As the conceptacles described by Collantes et al. (2002) as "immature" contain fully developed gametes and are very close (within hours or days) to becoming "fully mature", we considered these as reproductive. Also, since senescent individuals as described by Collantes et al. (2002) release very few gametes and the conceptacles are closed and moving to the medullar tissue (reverting to vegetative state), all senescent individuals were considered to be vegetative. In this manner, the study remained conservative as it is sometimes possible to find mature conceptacles within a generally senescent individual. Consequently, the two maturity stages "vegetative" and "reproductive" were distinguished as follows:

– Vegetative: Absence of cellular differentiation or initial differentiation of immature conceptacle between the subcortex and the medulla. In some cases there may be newly formed conceptacles but, as the gametes are undeveloped and the sex is unidentifiable, they were considered to be vegetative. Also, any senescent individual was equally categorised as vegetative.

– Reproductive: Presence of conceptacles that are well developed. These individuals have identifiable sexes where gametes may already be in the process of being released. The neck of the conceptacle, as well as the ostiole, may or may not be visible.

Colour determination

In order to examine whether colour is related to pigment concentrations, we determined the colour of each individual; a small piece of the blade (approximately 5×5 cm) was photographed and the picture was processed with the image analysis software Adobe Photoshop Lightroom 5.3. In this manner, the colour analysis remained objective and quantifiable, as opposed to a previous study where colour variation was only described qualitatively (Collantes et al. 1997), leading to possibly unreliable results based on the use of various systems and observers (Kendal et al. 2013). The blade pieces were photographed within 24 h after sampling in order to exclude the risk of colour variation caused by the drying of the samples. These pictures were taken in the laboratory and under constant light conditions to allow a more accurate comparison between images. Four study lamps with 4 W white and cold lightbulbs were used to illuminate the algae without creating shadows. The method used to quantitatively express the colouration of the images was red, green, and blue (RGB) values, which can define any given colour (Zhang et al. 2014). Although this is a rather common way of representing colour, it is important to note that RGB values are rarely standardised and vary according to the instrument used to obtain the image (Kendal et al. 2013). All images were taken using a Canon EOS Rebel T3i Camera at a distance of 50 cm from the sample and the RGB values of these samples were obtained using Adobe Photoshop Lightroom 5.3. The three colour values, R, G, and B, were evaluated individually. A high colour value means the colour is extremely light. Therefore, the RGB colour value 0,0,0 is associated with black. A perfectly white object has an RBG colour value of 255,255,255. As colour seemed to vary somewhat within a single sample, the average colour of nine different pixels within the sample was calculated.

Pigment concentrations

The studied pigments were Chl *a*, Chl *c* and total carotenoids, which includes mainly β-carotene and fucoxanthin. The method used to determine the pigment concentrations was as described in Tala et al. (2013). The samples were frozen at -80°C before being added to N,N-dimethylformamide (DMF) for 24 h at 4°C in darkness. As described in Rothäusler et al. (2011), the concentrations of Chl *a*, Chl *c* and total carotenoids were calculated using the dichromatic equations for Chl *a* (Inskeep and Bloom 1985), Chl *c* and carotenoids (Henley and Dunton 1995)

and expressed as mg per g wet weight. The absorbance of all pigment supernatants was measured in a UV-visible spectrophotometre (Rayleigh, model UV-1601, China) and at various wavelengths (480, 510, 664.5, 647 and 750 nm) in order to calculate each pigment concentration. Total pigment concentration, when used, was calculated by adding all individual pigments together and maintaining units as mg per g wet weight.

Phlorotannin concentrations

Phlorotannin levels were determined for a total of 24 samples for each location (eight vegetative samples, eight reproductive females, and eight reproductive males). Phlorotannin levels, as soluble phenols, were obtained using the method described by Koivikko (2008) with some moderate modifications. The standard used was purified phloroglucinol (Merck, Darmstadt, Germany). The frozen samples were lyophilised for 96 h before being pulverised with an Oster stick mixer. In order to extract the phenols, 10 mg of each sample was kept shaking for 24 h in 3 ml of 70% acetone (Merck, Darmstadt, Germany) at 4°C. The samples were then centrifuged at 3300 g for 15 min at 4°C and 250 µl of the supernatant was tested. To this sample we added 1250 µl of deionised water, 500 µl of 1 N Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany), and 1000 µl of 20% sodium carbonate ($NaCO_3$; Sigma-Aldrich, Steinheim, Germany). After addition of the reagents samples were incubated for 45 min at room temperature in complete darkness, and then centrifuged at 2000 g for 3 min at room temperature before reading the absorbance at 730 nm using a spectrophotometre (Rayleigh UV-1601 UV/VIS, Beijing, China). The results were expressed as percent dry weight. Two successive extractions were done 24 h apart and the two values were added to determine the final concentration.

Statistical tests

The number of stipes per holdfast was determined for all stranded and benthic samples. For the coalesced holdfasts, it was noted whether they hosted only vegetative (V), only reproductive males (M), only reproductive females (F), or both reproductive male and female stipes together (MF). In order to determine the probability that such a floating holdfast includes both sexes with a specific number of stipes, the obtained distribution of sexes in stranded and benthic samples was compared with a binomial distribution (presence of both sexes and absence of one or both

sexes) using a χ^2-test (Zar 2010). This analysis was done using Microsoft Excel 2013.

In order to determine the relationship between pigments and the coloration of the kelp (R, G, and B, separately) for benthic samples from Puerto Oscuro and Totoralillo Sur, the Pearson correlation coefficient was calculated, as well as ANOVA of simple linear regression to verify the significance of the slope. To compare concentrations of all the studied pigments and phlorotannins with the sexual stage (fixed factor: male, female, or vegetative) and according to their localities (fixed factor: Puerto Oscuro, Totoralillo Sur), a two-way ANOVA was applied after previous analyses of normality (Kolmogorov-Smirnov's test) and homoscedasticity (Levene's test). All pigment values were transformed using $\log_{10}(x+1)$ and phlorotannin values were modified using the arcsine transformation. Lastly, if there were significant effects, the differences between treatments were examined *a posteriori* with Tukey HSD tests (Zar 2010). For all analyses with pigments, three stipes were considered to be outliers and were therefore excluded from the tests. All statistical tests were done with R 3.1.3, using the lme4 package (R Development Core Team 2015).

Results

Stipe distribution on holdfasts

Based on the samplings of rafting (recently stranded) holdfasts from the winters 2013, 2014 and 2015, 52% of the 1044 holdfasts had only one stipe, the majority of which were in the vegetative state (Figure 2A). Of all stranded holdfasts, only 5.7% had at least one stipe of each sex that was reproductive (Figure 2A). Results were similar for the benthic holdfasts from winter 2015 as most holdfasts only had one stipe. However, in this case stipes were mostly in the reproductive stage, whether male or female, and 17.7% of all holdfasts had reproductive stipes of both sexes (Figure 2B). The distribution of holdfasts with both sexes were significantly different from a binomial distribution both for stranded (χ^2=23.35; df=13; p<0.05) and for benthic holdfasts (χ^2=14.55; df=7; p<0.05).

Pigments and colour

The total pigments were negatively correlated with both the R (r=-0.205; p=0.003) and G (r=-0.159; p=0.022) colour values (Table 1). There was no relationship between Chl
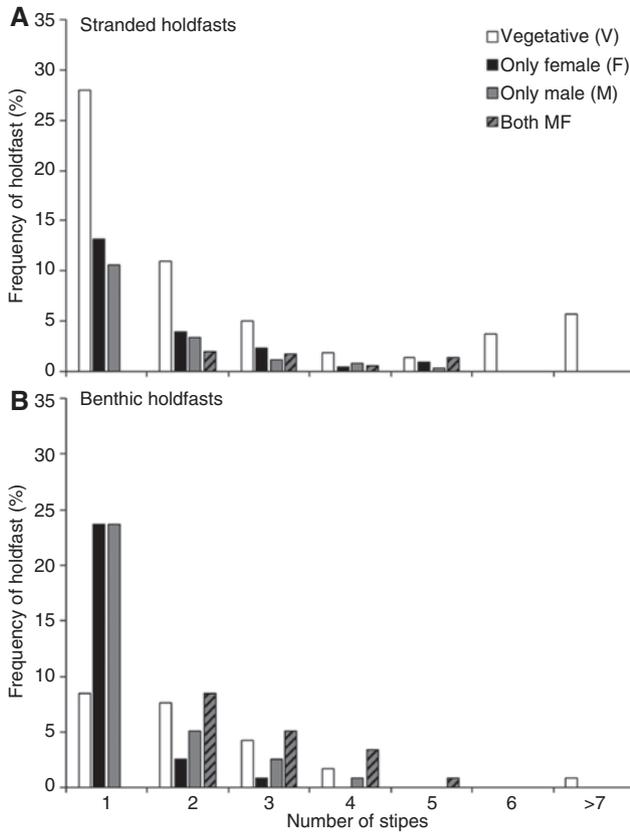
Figure 2: *Durvillaea antarctica*: frequency of holdfasts with different numbers of stipes, and with only vegetative stipes, only male stipes, only female stipes, and both sexes within one holdfast. Samples collected at (A) 33 beaches on the coast of Chile between 28°S and 42°S (n=1044 stranded holdfasts during the winters 2013, 2014 and 2015), and (B) two sites near the northern distribution limit of *D. antarctica* (n=56 holdfasts from Puerto Oscuro and n=62 holdfasts from Totoralillo Sur).

Table 1: *Durvillaea antarctica*: summary of correlations and ANOVAs of simple linear regression between pigments and colour values R (red), G (green), and B (blue) in the blades of the different stipes samples.

Pigments	Colour	r	df	F	p-Value
Total pigment	R	-0.205	1;203	8.93	**0.003**
	G	-0.159	1;203	5.28	**0.022**
	B	0.013	1;203	0.03	0.844
Chlorophyll *a*	R	-0.212	1;203	9.58	**0.002**
	G	-0.164	1;203	5.66	**0.018**
	B	0.008	1;203	0.01	0.908
Chlorophyll *c*	R	-0.162	1;203	5.49	**0.020**
	G	-0.120	1;203	2.97	0.086
	B	0.045	1;203	0.42	0.516
Carotenoids	R	-0.205	1;203	8.94	**0.003**
	G	-0.163	1;203	5.54	**0.019**
	B	0.006	1;203	0.01	0.926

Significant values (p<0.05) in bold face.
Stipes from both sites (Puerto Oscuro and Totoralillo Sur) were pooled for these analyses.

a, Chl *c*, carotenoids and total pigment concentrations and the blue colour. Statistically significant colour differences were observed between the sexual stages, where the vegetative stipes had higher red, green, and blue colour values than female stipes (Figure 3). However, the male stipes did not differ from either vegetative or female stipes (p<0.05), except for the blue colour value where values of males were lower than those of vegetative individuals (Figure 3). Furthermore, no location effect was found for the red and green colour values, but the blue colour values were higher in Puerto Oscuro than in Totoralillo Sur (Figure 3; Table 2). Also, there was no significant interaction between localities and sexual stages (Table 2).
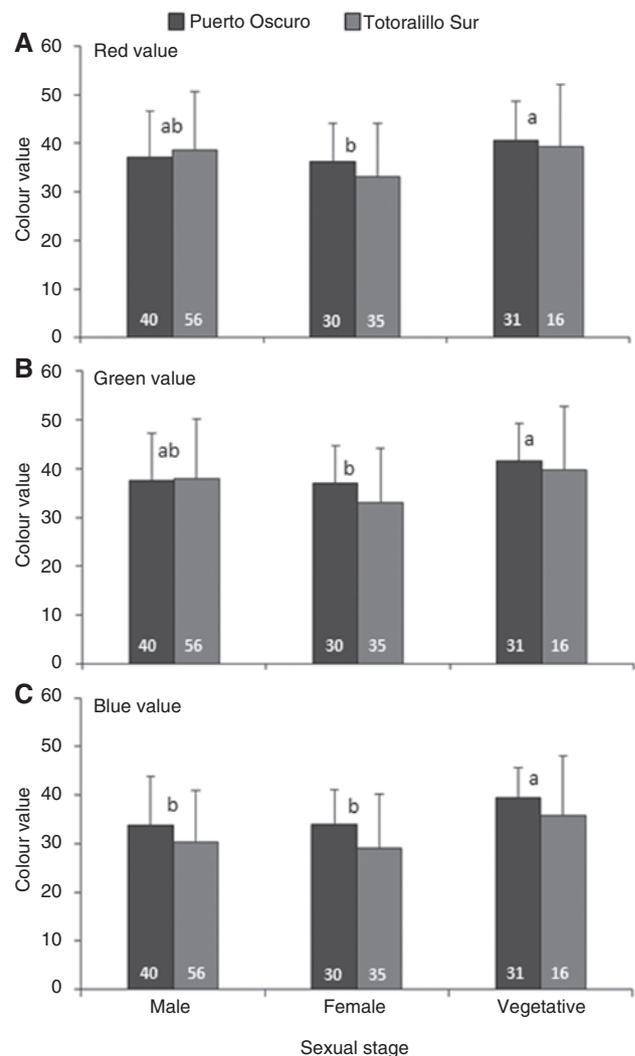

Figure 3: *Durvillaea antarctica:* average (mean±SD) colour values of algal samples of different sexual stages and from two localities, Puerto Oscuro and Totoralillo Sur. Different letters above the columns indicate differences between sexual stages significant at p=0.05. Numbers of stipes from each site and sexual stage are listed at the bottom of each column.

Table 2: *Durvillaea antarctica*: summary of two-way ANOVAs for colour (R, G and B) according to site (Puerto Oscuro and Totoralillo Sur) and sexual stage (vegetative, reproductive male, and reproductive female) of algal samples.

Variable	Effect	df	F	p-Value
Red value, R	Site (S)	1;202	0.41	0.522
	Stipe stage (SS)	2;202	3.49	**<0.05**
	S×SS	2;202	0.95	0.390
Green value, G	Site (S)	1;202	1.33	0.251
	Stipe stage (SS)	2;202	3.68	**<0.05**
	S×SS	2;202	0.82	0.440
Blue value, B	Site (S)	1;202	7.58	**<0.01**
	Stipe stage (SS)	2;202	5.92	**<0.01**
	S×SS	2;202	0.12	0.888

Significant values (p<0.05) in bold face.

Pigments, location, and sexual stage

For Chl *a*, Chl *c* and carotenoids, there was a significant difference in pigment concentration between vegetative and reproductive individuals, whether male or female. Vegetative individuals had significantly higher concentrations of total and specific pigments than reproductive individuals, although carotenoids for female stipes have similar levels as vegetative stipes (Figure 4). There was no difference in concentrations between males and females, but pigment concentrations were consistently higher in Puerto Oscuro than in Totoralillo Sur. However, there was no significant interaction between site and stipe stage (Table 3).

Phlorotannins, location, and sexual stage

Phlorotannin concentrations were significantly higher in Totoralillo Sur than in Puerto Oscuro, although there was no difference for females (Figure 5). Furthermore, phlorotannin concentrations of female individuals were significantly higher than those of vegetative individuals in Puerto Oscuro (Figure 5). There was no significant interaction between the site and stipe stage (Table 3).

Discussion

The current study has confirmed that reproductive individuals of *Durvillaea antarctica* do in fact travel together via coalesced holdfasts. Additionally, there is no significant biochemical difference between reproductive males and females when it comes to Chl *a*, Chl *c*, total carotenoids, and phlorotannins. These results indicate that the
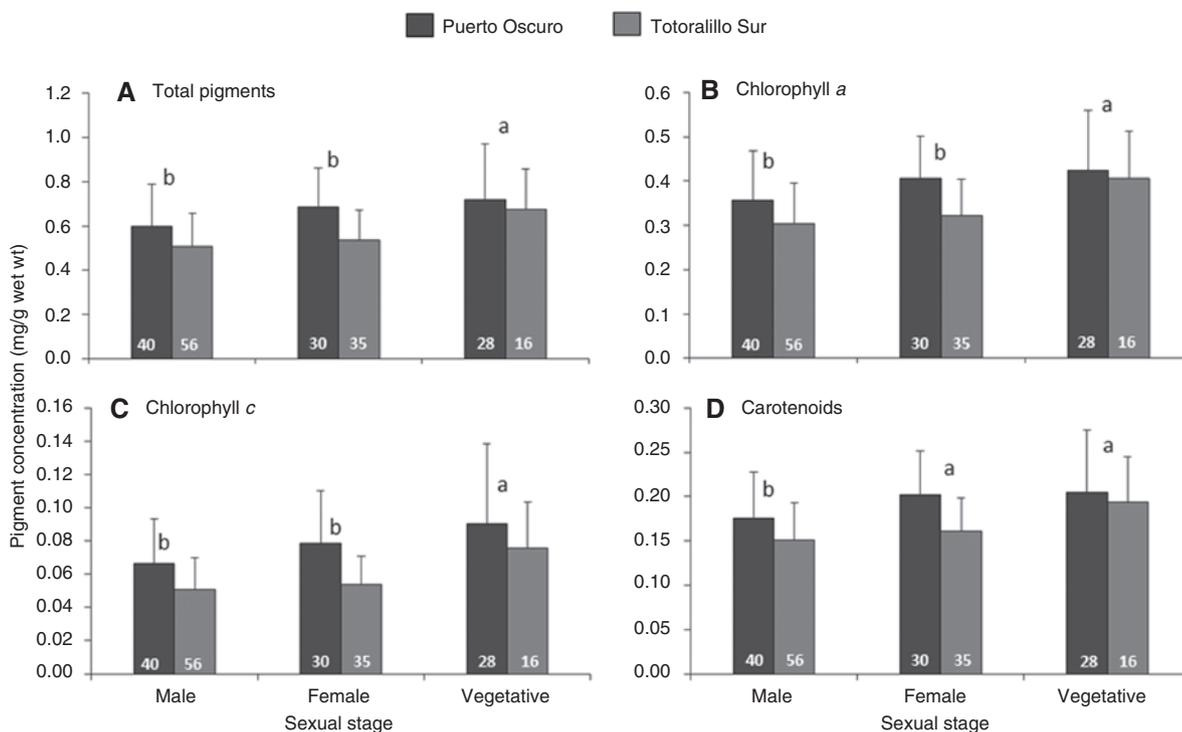

Figure 4: *Durvillaea antarctica*: average (mean±SD) concentration (mg/g wet wt) of pigments in blade samples of different sexual stage and locality, Puerto Oscuro and Totoralillo Sur. Different letters above the columns indicate differences between sexual stages significant at p=0.05. Numbers of stipes from each site and sexual stage are listed at the bottom of each column.

Table 3: *Durvillaea antarctica*: summary of two-way ANOVAs for variables (pigments and phlorotannins) according to site (Puerto Oscuro and Totoralillo Sur) and sexual stage (vegetative, reproductive male, and reproductive female) of algal samples.

Variable	Effect	df	F	p-Value
Total pigment	Site (S)	1;199	11.67	**<0.001**
	Stipe stage (SS)	2;199	8.97	**<0.001**
	S×SS	2;199	1.24	0.289
Chlorophyll *a*	Site (S)	1;199	10.35	**<0.001**
	Stipe stage (SS)	2;199	9.10	**<0.001**
	S×SS	2;199	1.31	0.272
Chlorophyll *c*	Site (S)	1;199	18.48	**<0.001**
	Stipe stage (SS)	2;199	10.33	**<0.001**
	S×SS	2;199	0.59	0.555
Carotenoids	Site (S)	1;199	11.69	**<0.001**
	Stipe stage (SS)	2;199	7.89	**<0.001**
	S×SS	2;199	1.27	0.283
Phlorotannins	Site (S)	1;42	7.04	**<0.05**
	Stipe stage (SS)	2;42	3.53	**<0.05**
	S×SS	2;42	1.64	0.205

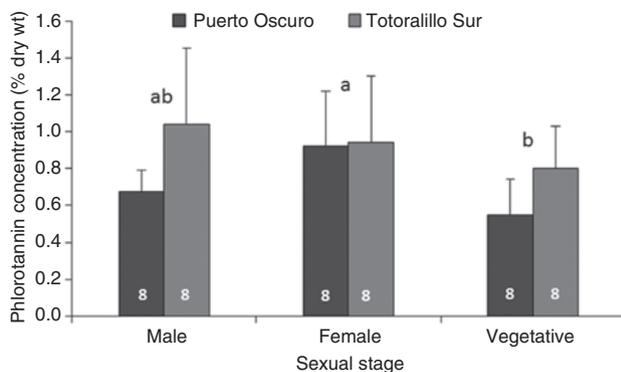Significant values (p<0.05) in bold face.

Figure 5: *Durvillaea antarctica*: average (mean±SD) concentration (% dry wt) of phlorotannins in blade samples of different sexual stage and locality, Puerto Oscuro and Totoralillo Sur. Different letters above the columns indicate differences between sexual stages significant at p=0.05. Numbers of stipes from each site and sexual stage are listed at the bottom of each column.

lack of genetic connectivity of *D. antarctica* populations along the continental coast of Chile is not a result of males and females failing to disperse together.

Dioecious species, but united in holdfasts

It has been extrapolated that within the entire Southern Ocean, over 70 million *Durvillaea antarctica* kelp rafts are afloat at any one time (Smith 2002). Furthermore, around 20 million of these rafts are estimated to have a

holdfast (Smith 2002), potentially containing both sexes. Also along the Chilean coast, large numbers of *D. antarctica* are floating at any given moment in time (Hinojosa et al. 2011), many of which have intact holdfasts (Tala et al. 2013, this study). Of the 1000 recently stranded individuals of *D. antarctica* with holdfasts, almost half had a coalesced holdfast with at least two stipes. Thus, the probability of long-distance dispersal is greatly enhanced as the presence of multiple individuals in a single holdfast substantially improves the possibility that individuals of both sexes are travelling together over great distances, although the overall proportion of holdfasts with mature individuals of both sexes is relatively small (5–17%).

It is likely that holdfast coalescence in *D. antarctica* is responsible for the impressive long-distance dispersal of this species throughout the subantarctic region (Fraser et al. 2010b). In fact, other seaweeds that are capable of long-distance dispersal, such as *Macrocystis pyrifera* (Linnaeus) Agardh 1820 (González et al. 2015) and *Fucus vesiculosus* (Malm and Kautsky 2004), have also been proven to have coalesced holdfasts, contributing to the idea that holdfast coalescence may play a crucial role in the dispersal of certain species, particularly if they are dioecious.

The small proportion of *D. antarctica* holdfasts travelling with both sexes may be further constrained by another important condition, the timing of maturity of the individuals of both sexes. As *D. antarctica* tends to mature over a rather extensive time period of 3 months between May and July (Santelices et al. 1980), it could also be possible that not all individuals within a holdfast are fully mature at the same time. This relatively long reproductive season and a generally simultaneous release of gametes within each individual limits the probability that male and female gametes are released at the same time, therefore diminishing fertilisation probabilities. Furthermore, it is only during this fertile phase that *D. antarctica* rafts containing both sexes can contribute to the genetic connectivity between populations. As the benthic individuals had a higher proportion of reproductive individuals than the stranded ones, which were mainly vegetative, gamete release might occur before floating individuals return to the shore thereby further limiting their dispersal potential.

Biochemical composition

The results from Collantes et al. (1997) indicated that, in *Durvillaea antarctica*, there might exist a correlation between the colour of an individual and its sex. They reported that lighter, yellower individuals were mostly males whereas darker, blacker individuals were generally

females. Such results suggest sexual dimorphism for *D. antarctica*, meaning there are phenotypic differences between the males and females, which would possibly be caused by the fact that reproductive females carry millions of large, dark eggs in the conceptacles whereas male antheridia are small and light. However, the results of the current study did not confirm any sex-specific colour differences and did not allow determining whether eventual colour difference might be due to the eggs.

Using RGB values to describe the colours, total pigments and each individual pigment were found to have an effect on both the R and G colour values and none on the B colour value (Table 1). However, for both R and G, the effects of pigment concentrations on the colour pattern were relatively minor. It is possible that another pigment component could play a more central role in determining the colour of *D. antarctica*. This could particularly be the case for carotenoids, as there are many types of carotenoids and some of them are continuously adjusted as a result of photoprotective responses (Karsten 2008). As for what can be explained by the pigments, it was found that there was a small negative relationship between total pigments and both the R and G colour values. Visually, this means that when an individual has a higher pigment concentration, it will be darker. One may therefore assume that (the darker) females would have higher pigment concentrations than males. However, this was not the case in our study as reproductive males and females had similar pigment concentrations.

Vegetative individuals had a significantly higher concentration of pigments than reproductive individuals, male or female, and both sexes were relatively similar in concentration. Though no cause was identified, it is possible that vegetative bull kelps, with a full cortex without colourless conceptacle cells, have higher pigment concentrations. Phlorotannins are known to serve a protective role (Li et al. 2009) and higher concentrations of phlorotannins within female tissues may serve to protect the gametes of the females. Sexual dimorphism in chemical concentrations has been demonstrated in other species, such as *Gracilaria chilensis* (Guillemin et al. 2014). For *D. antarctica*, no significant difference was found between males and females, which could indicate that both sexes use phlorotannins equally for protection. Phlorotannins are known to act as antioxidants, participate in UVR protection, and in general defence (Karsten et al. 2009, Wang et al. 2009, Onofrejová et al. 2010, Steinhoff et al. 2012). As we found no sex-specific differences in the concentration of these biochemical compounds it seems that both sexes of *D. antarctica* are equally fit to survive rafting along the coast of central Chile.

It is interesting to note that the specimens from Puerto Oscuro, which had higher pigment concentrations than the samples from Totoralillo Sur, were collected from a site that receives very little direct sunlight under a high cliff. Possibly, these bull kelps had adjusted pigment concentrations to low light conditions as also reported for other species (Colombo-Pallota et al. 2006, Hanelt and Figueroa 2012). As was the case for pigments, there was a significant difference in phlorotannin concentrations between the sites. However in this case, phlorotannin levels were higher in Totoralillo Sur. Increases in phlorotannin concentrations have been related to changes in PAR and UVR levels (Swanson and Druehl 2002, Cruces et al. 2013) and herbivore pressure (Van Alstyne 1988, Pavia and Toth 2000). Thus, site-specific environmental conditions cause variation in pigments and phlorotannin concentrations in *D. antarctica* and appear to be more important than sex-specific differences. This could suggest that kelps from some sites are better suited for long-distance rafting than those from other sites. In future studies it would be interesting to study other components that could possibly have an impact on gamete survival during rafting dispersal, such as carbohydrates or proteins, which might differ between sexes (Jones 1957) and sexual stages (Skriptsova et al. 2012).

Conclusions and outlook

In this study, it has been demonstrated that reproductive individuals of both sexes of *Durvillaea antarctica* travel together in coalesced holdfasts. Furthermore, there is no significant difference between reproductive males and females in essential biochemical compounds that could result in sex-specific differences in the potential for rafting dispersal. Thus, the phytogeographic pattern of *D. antarctica* along the continental coast of Chile (Fraser et al. 2010b) does not appear to be due to biological constraints limiting the dispersal potential of this floating kelp. Instead, it seems more likely that the process that limits the dispersal of species such as *D. antarctica* is an ecological or physical one. For instance, density-blocking could potentially influence population connectivity in a variety of species (Waters et al. 2013, Neiva et al. 2014). Also, prevailing winds and currents (e.g. Humboldt Current System) can greatly influence the dispersal trajectories of algal rafts (Rothäusler et al. 2015), possibly causing certain areas of the coast to receive less input. Future studies should concentrate on studying the impact of density-blocking and currents on the population connectivity of rafting seaweed species.

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