

Blades and papillae as likely dispersing propagules in Chilean populations of *Mastocarpus* sp. (Rhodophyta, Gigartinales)

Láminas y papilas como posibles propágulos de dispersión en poblaciones chilenas de
Mastocarpus sp. (Rhodophyta, Gigartinales)

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Resumen.- *Mastocarpus* sp. es un alga roja nativa a la costa del Pacífico de Norteamérica, y también presente en Concepción, Chile (ca. 37°S), donde se ha propuesto como especie no indígena. Su establecimiento y expansión podrían verse favorecidos si láminas y papilas constituyeran propágulos. Las características de desprendimiento de estas dos estructuras apoyan esta interpretación. Láminas de *Mastocarpus* sp. requirieron menos fuerza para ser desprendidas desde las rocas que láminas de otras algas rojas comunes. Asimismo, un estimado mensual de 14,9% de las papilas con cistocarpos maduros se desprenden de láminas sometidas a turbulencia en laboratorio. Además, es común encontrar láminas y papilas a la deriva, y liberan esporas de pigmentación normal. La distribución de papilas a lo largo de las láminas, considerando su madurez, sugiere un patrón de desprendimiento predecible. Papilas nuevas fueron más abundantes en segmentos distales de láminas, y papilas viejas en segmentos centrales. La abundancia de cicatrices dejadas por papilas desprendidas fue mayor en segmentos basales, sugiriendo que las papilas que dejaron esas cicatrices portaban cistocarpos maduros. Concluimos que los puntos de adhesión de láminas y papilas se tornarían relativamente más débiles a medida que crecen, facilitando su desprendimiento. Esto no representa la abscisión de estructuras senescentes ya que en su interior crecen carposporofitos con esporas que pueden ser liberadas. Esto facilitaría el establecimiento de nuevos individuos a distancias y lugares diferentes de los que alcanzarían las esporas, pudiendo ser consideradas como estructuras alternativas de dispersión. Como tales, podrían afectar la dinámica poblacional y expansión de rango de *Mastocarpus* sp. en Chile.

Palabras clave: Desprendimiento, especie no indígena, estrategia de dispersión, fragmentos a la deriva, Phylloporaceae

Abstract.- *Mastocarpus* sp. is a red macroalga native to the Pacific coast of North America, and present around Concepción, Chile (ca. 37°S), where it has been suggested as a non-indigenous species. Its establishment and expansion could be favored if blades and papillae function as propagules. The characteristics of the detachment of these two structures support this interpretation. Blades of *Mastocarpus* sp. become detached from rocks more readily than other common, bladed red seaweeds. In turn, a monthly estimate of up to 14.9% of papillae carrying mature cystocarps easily broke off from blades when experimentally subjected to turbulence in the laboratory. Additionally, blades and papillae are commonly found drifting, and release normally pigmented spores. The distribution of papillae along the blades, considering their maturity, suggests a predictable detachment pattern. New papillae were more abundant on distal segments, old papillae on middle segments, and scars of shed papillae on basal segments, suggesting that shed papillae carried mature cystocarps. We conclude that blades and papillae have attachment points that become relatively weaker as they grow and mature, facilitating their detachment. This does not represent abscission of empty or senescent structures because carposporophytes growing inside these structures still carry spores that can be released. Thus, blades and papillae may be important in facilitating the establishment of new individuals at distances and places different from what is achieved by spores and, hence, could be considered as alternative dispersing entities. As such, they could affect the population dynamics and range expansion of *Mastocarpus* sp. in Chile.

Key words: Detachment, dislodgment, dispersal strategy, drifting fragments, non-indigenous species, Phylloporaceae

INTRODUCTION

For seaweeds, and other benthic organisms, dispersal is a fundamental process in maintaining and restoring local populations, and in the colonization of new environments (Santelices 1990, Reed *et al.* 2000, Kinlan & Gaines 2003, Shanks *et al.* 2003). In seaweeds, propagules are structures that become detached from the parental thallus, drift away and produce new established individuals (Hoffmann 1987, Macchiavello *et al.* 2003, Hernández-Carmona *et al.* 2006, Stewart 2006, Sussmann & DeWreede 2007, McKenzie & Bellgrove 2008).

Unicellular entities (*e.g.*, spores, gametes and zygotes) are the most common types of propagules in seaweeds, and have received considerable attention in the literature (*e.g.*, Hoffmann 1987, Santelices 1990, Clayton 1992, Norton 1992, Reed *et al.* 1992, Kendrick & Walker 1995, Gaylord *et al.* 2002, Opazo & Otaíza 2007). In contrast, the importance of multicellular structures associated with vegetative reproduction is less frequently considered, despite the fact that breakage and drifting of thallus fragments may be a common dispersal mechanism for many seaweeds. Moreover, unicellular propagules have the capacity for primary attachment via the evacuation of adhesive vesicle contents, whereas drifting fragments may have different secondary attachment mechanisms, *e.g.*, rhizoids (Pueschel 1990, Salinas 1991) or other secondary attachment structures (*e.g.*, Otaíza & Fonseca 2011).

Detachment and drifting of large fragments (*e.g.*, sporophylls in some Laminariales) or even whole thalli carrying mature reproductive structures is frequent in macroalgae (Vásquez & Tala 1995, Mathieson *et al.* 2003, Macaya *et al.* 2005, Hernández-Carmona *et al.* 2006, McKenzie & Bellgrove 2008). While drifting, or once in contact with the substratum, these fragments may release spores or gametes, which are able to generate new individuals.

The release and dispersal of vegetative fragments can have important demographic effects. For example, fragments of *Gracilaria chilensis* are the main propagation structures for this species (Santelices & Doty 1989, Buschmann *et al.* 1995). Drifting can also have important biogeographic effects. Floating kelp contribute to the connectivity of their populations and of associated organisms (Smith 2002, Hinojosa *et al.* 2010). Furthermore, fragmentation has favored the expansion of some non-indigenous algal invaders (Maggs & Stegenga 1999) like *Caulerpa taxifolia* in Mediterranean and Australian

waters (Ceccherelli & Cinelli 1999, Smith & Walters 1999, Ceccherelli & Piazzini 2001, Wright & Davis 2006, Khou *et al.* 2007) and *Codium fragile* on Atlantic and Pacific coasts (Garbary *et al.* 2004). Indeed, invading species increase their chances of becoming established and expanding their populations when they have multiple types of propagules.

The genus *Mastocarpus* (Rhodophyta, Gigartinales) has several species on the northeastern Pacific coasts (Lindstrom *et al.* 2011). In contrast, a single species has been cited for the Chilean coasts (southeastern Pacific). Initially identified as *Mastocarpus papillatus* (C. Agardh) Kützing, the species present in Chile has been recently reassigned to *Mastocarpus latissimus* (Harvey) (see Lindstrom *et al.* 2011). This species is distributed from Alaska to southern California (Lindstrom *et al.* 2011) and is also very common around Concepción, south-central Chile (36°45'S; 73°03'W; Santelices 1989, Hoffmann & Santelices 1997, cited in both references as *M. papillatus*). This disjunct geographic distribution, together with a lack of congeners in Chile and multiple congeners present in Europe and Asia, occurrence in regions with potential vectors (*e.g.*, international and national ports), and biological characteristics common in successful algal invaders like crustose growth form and ability for asexual reproduction (Hewitt *et al.* 2007, Carlton 2009), has led to the proposal that this species is introduced on Chilean coasts (Castilla *et al.* 2005).

Species in the genus *Mastocarpus* have heteromorphic life-cycles in which crustose tetrasporophytes alternate with bladed gametophytes (Zupan & West 1988, Lindstrom *et al.* 2011), although direct development of female gametophytes also occurs (Zupan & West 1988, Fierst *et al.* 2010, Lindstrom *et al.* 2011). In the *Mastocarpus* populations present in Chile, blades grow from a perennial, crustose base to which they are attached by a narrow stipe (Hoffmann & Santelices 1997). Reproductive, female gametophytic blades produce papillae which harbor the carposporophytes (Lindstrom *et al.* 2011). Unlike *M. latissimus*, male gametophytes are smooth, without papillae; given this discrepancy with the description given by Lindstrom *et al.* (2011), the species studied in this work will be referred to as *Mastocarpus* sp. For *Mastocarpus* species, only unicellular propagules (tetraspores and carpospores) have been described (*e.g.*, West 1972, Polanshek & West 1977, Zupan & West 1988), although the possibility of detached blades playing a role in dispersal has been suggested (*e.g.*, Carrington 1990).

Preliminary observations indicate that the stipes of the blades and the peduncles of the papillae break off easily, even before many of the spores they bear are released (R. Otaíza, pers. observ.). The aim of this study is to evaluate the possibility that detachment of papillae and of blades carrying mature papillae may be important as an alternative dispersal mechanism of this species. We evaluated some detachment characteristics of blades and papillae, the occurrence of these structures drifting in the field, and the likelihood of spore release. We also evaluated whether there was a consistent pattern of papillae shedding along the blades in relation to their maturity, as a possible strategy to release papillae carrying viable spores.

MATERIALS AND METHODS

STUDY SITES

Fronds of *Mastocarpus* sp. were collected from three localities in south-central Chile: Península Hualpén (36°48'S; 73°10'W), El Morro (36°31'S; 73°57'W) and Coliumo (36°31'S; 72°57'W). All include moderately sheltered rocky shores where *Mastocarpus* sp. is abundant. The *Petrocelis* stage can be commonly found in most localities. Another site, Lenga (36°45'S; 73°10'W),

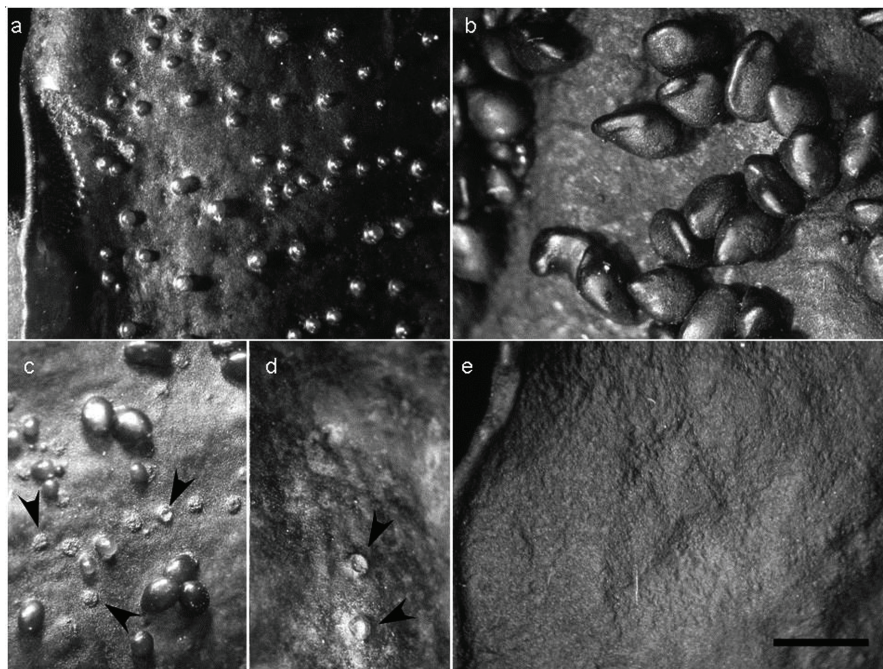
was used to deploy some devices due to accessibility and protection offered by the Abate Juan Ignacio Molina Coastal Marine Laboratory (Universidad Católica de la Santísima Concepción).

DISTRIBUTION OF PAPILLAE AND SCARS ALONG THE BLADES

To determine the distribution of papillae along the blades, female blades were collected at Península Hualpén in April and May, 2007. In the laboratory, blades with a complete apex, were sorted according to the length of the longest axis into three size-categories: small (5.0-6.9 cm in length), medium (7.0-8.9 cm) and large (9.0-10.9 cm). A total of 18 blades of each size-category were analyzed by cutting consecutive, 2 cm long segments, starting from the base and extending along the longest axis, resulting in 3, 4 or 5 segments, depending on the size-category.

Papillae were classified into two categories according to their size and development (Fig. 1). New papillae were up to 1 mm in length, with the base wider than the tip, and no distinguishable swelling of a developing carposporophyte inside. Old papillae were those with a narrow peduncle, presenting a distinguishable swelling resulting from the presence of developing carposporophytes. A third category included papillae that had broken off, identified as a circular scar on the blade surface or as the remains of the peduncle.

Figure 1. Surface of the blades of female (a-d) and male (e) gametophytes of *Mastocarpus* sp. Female gametophytes have new papillae (a) and old papillae (b). Papillae that have fallen off leave circular scars (arrowheads in c) or the remains of the peduncule of the papillae (arrowheads in d). Blade material collected at Lenga, March 2012. All photographs have the same scale bar: 5 mm, included in (e) / Superficie de láminas de gametofitos femeninos (a-d) y masculino (e) de *Mastocarpus* sp. Los gametofitos femeninos presentan papilas nuevas (a) y papilas viejas (b). El desprendimiento de papilas deja cicatrices circulares (cabezas de flecha en c) o los restos de los pedúnculos de las papilas (cabezas de flecha en d). Láminas colectadas en Lenga, marzo 2012. Todas las fotos tienen la misma barra de escala: 5 mm, incluida en (e)



For each segment, the numbers of new and old papillae and scars were counted under a dissecting microscope, in two fields of view along each side of the blade. Values were averaged for each segment, separately for each type of reproductive structure. Data were standardized to one cm². Density of new and old papillae and scars was analyzed separately for each frond size-category using an unreplicated randomized block ANOVA design, with Segment as the main factor and Blade as the block factor. To assure independence among density values in relation to blades, data for the blades of each size-category were randomly separated in three groups of six blades. Analyses for each of the three types of reproductive structures were done with data from one of these three groups of blades.

Cochran's test was used to evaluate the assumption of homogeneity of variances, and Shapiro-Wilk's test was used to evaluate the assumption of normality for all ANOVAs. Square-root or logarithmic transformations (Zar 1999) successfully corrected non-compliance with these assumptions when they occurred, and angular transformations corrected heterocedasticity of data expressed as proportions (see below). Significant differences detected by the analyses were explored with Tukey's HSD *a posteriori* test.

DETACHMENT OF PAPILLAE IN LABORATORY CONDITIONS

To evaluate whether detachment of papillae was related to blade length, blades were collected from El Morro between April and May, 2007, and sorted into the three blade size-categories. A fourth category, large tattered blades, included large blades (9.0-10.9 cm in length) whose apical portion was missing, indicating that these blades were originally longer than blades in the other categories. For each category, groups of blades were standardized to 5 g, and the total number of old papillae in each group was counted. Three replicate groups of blades were used in each run of the experiment and size-category. Blade groups were placed in 500 mL graduated cylinders filled with seawater at 12 ± 1°C. Double exit air pumps for aquaria were used to pump air into the graduated cylinders to produce moderate turbulence. After 24 h, fronds were removed from the container and the detached papillae were counted. Each papilla was cut open to check for the presence of developing carposporophytes. The entire procedure was repeated on three occasions. The initial number of papillae and percentage of detached papillae relative to the initial number, were analyzed using two-way ANOVAs with Blade Size-category as a fixed effect factor, and Version of the Experiment as a random effect factor.

DETACHMENT OF BLADES

When collecting seaweeds in the field, the blades of *Mastocarpus* sp. detach more easily than those of other common red, intertidal seaweeds of similar size. To validate this observation we evaluated the force required to remove individual fronds of *Mastocarpus* sp. and two other common bladed red seaweeds, *Mazzaella laminarioides* and *Nothogenia fastigiata*, at Península Hualpén, in October 2006. For the three species, tested blades ranged between 10 and 15 cm in length. Blades of all species were sampled from the same patches (*i.e.*, within 2 m) on rocky surfaces with the same orientation, slope and exposure to wave action. To measure the force required to detach the blades, each blade was individually rolled, from the tip to about 3 cm from the base, onto a ballpoint pen casing, and fastened with clamps. The ends of this tube were attached to a 10 N dynamometer. Once secured, blades were pulled perpendicularly from the substratum with a steady force that produced detachment within about one second. Only cases in which breakage occurred from the base of the blades were recorded. For each species 100 trials were conducted. Results were compared using one-way ANOVA.

DRIFTING PAPILLAE

The occurrence of drifting papillae was assessed using trapping devices in the rocky intertidal zone at Lenga, where *Mastocarpus* sp. is present in dense patches. Each device consisted of a vertically oriented, 110 mm diameter, 40 cm long PVC tube, with a 1 cm mesh-covered top, closed at the bottom, and a lateral drainage window covered with 500 µm mesh (Oróstica 2008). The upper end of the tube-trap was removable. It had a one-way entrance made of a 110-75 mm reducer coupling. The wider outer opening of the coupling was covered with a perforated cap (opening area: 78.5 cm²), which was covered with a plastic 1 cm mesh to prevent larger drifting seaweeds from being trapped or blocking the entrance. The narrower inner opening of the coupling was fitted with a mesh cone that worked as a one-way valve to prevent loss of the collected material. Devices were encased in aluminum strip harnesses and anchored to boulders in the low intertidal zone using bungee-cords. On three occasions between April and May, 2007, three devices were continuously deployed for two days. Collected material was sorted and papillae were counted. Of the local species, *Mastocarpus* sp. is the only one that has clearly pedunculated papillae; in other species, prominent cystocarps have a broad connection to the

female thallus. As in the laboratory experiment, collected papillae were cut open to observe developing carposporophytes. The average of the daily mean number of positively identified papillae was compared to the null expectation of zero using Student's t-test.

SPORE RELEASE BY DRIFTING BLADES

Drifting *Mastocarpus* sp. blades were collected by hand from the water's edge along a 100 m transect parallel to the coast at Coliumo in March 2009. Only those with healthy appearance were collected and transported to the laboratory. To simulate the effect of desiccation at low tide, fronds were rinsed in seawater and exposed to air for 3 h. Fronds were then placed individually in 400 mL storage dishes with filtered seawater and maintained, at $13 \pm 1^\circ\text{C}$. After 24 h, fronds were removed and water samples were checked for the presence of spores.

RESULTS

DISTRIBUTION OF PAPILLAE AND SCARS ALONG THE BLADES

The density of new and old papillae and scars differed among blade segments, each presenting a distinct pattern. These trends were consistent among blades from the three size-categories. For new papillae, significant differences were found among segments for all size-categories (ANOVA Segment: $F_{(2,10)} = 9.32, P < 0.0005$; $F_{(3,15)} = 8.43, P < 0.002$; $F_{(4,20)} = 17.43, P < 0.0001$, for small, medium and large blades, respectively). For all size-categories, Tukey's tests indicated that new papillae were more abundant in the distal segment than the basal segment (Fig. 2). Thus, new papillae were most abundant close to the growing tip of the blade, and their density greatly decreased towards the older parts at the base of the blades.

Density of old papillae differed significantly among segments for medium and large blades (ANOVA Segment: $F_{(3,15)} = 4.05, P = 0.027$; $F_{(4,20)} = 3.65, P = 0.022$, for medium and large blades, respectively). For medium blades, Tukey's test indicated that only the highest density, obtained in the third segment, differed from the lowest density, found in the basal segment; other comparisons were not significant. For large blades, the density of old papillae was greater in the third and fourth segments than in the first, other comparisons were not significant. For small blades, the density of old papillae ranged from $37.2 \pm 3.6 \text{ cm}^{-2}$, in the basal segment, to $49.0 \pm 2.8 \text{ cm}^{-2}$, in the second segment, but no differences among segments were found (ANOVA Segment: $F_{(2,10)} = 2.19, P = 0.16$).

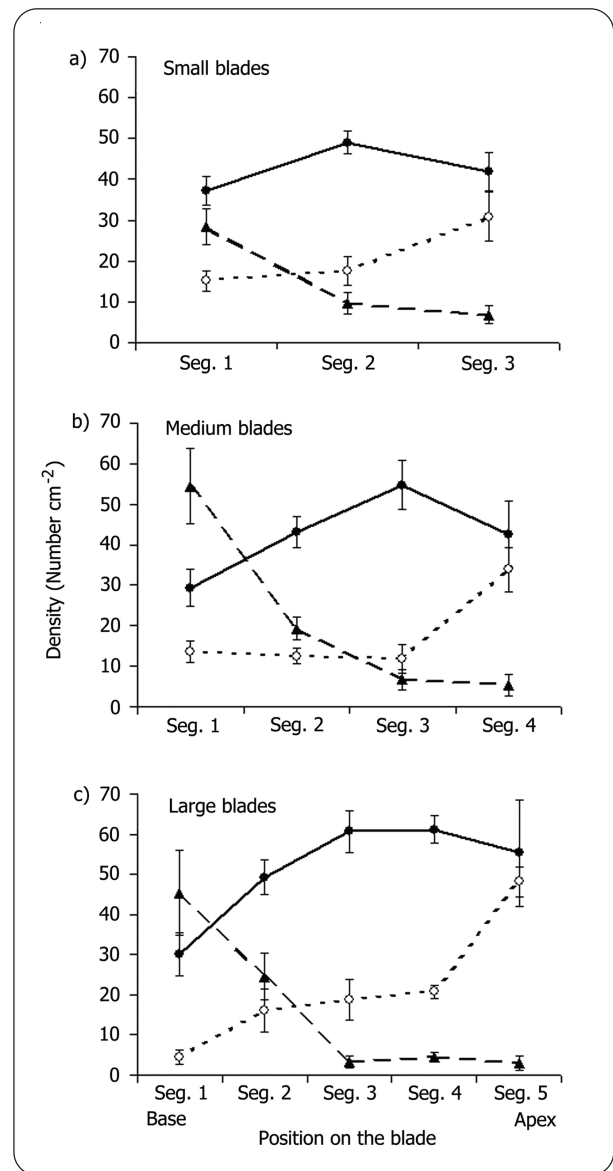


Figure 2. Mean density (\pm standard error) of new papillae (open circles, dotted line) and old papillae (full circles, continuous line) and scars left by shed papillae (triangles, dashed line) on blades of *Mastocarpus* sp. Whole blades (*i.e.*, with an intact apex): a) small blades (5.0-6.9 cm in length), b) medium blades (7.0-8.9 cm) and c) large blades (9.0-10.9 cm), considering six different blades for each stage of development of the reproductive structures. Blade segments: Seg. 1 (basal) to Seg. 5 (apical in large blades) / Densidad promedio (\pm error estándar) de papilas nuevas (círculos abiertos, línea punteada) y papilas viejas (círculos cerrados, línea continua) y cicatrices dejadas por papilas desprendidas (triángulos, línea segmentada) en láminas de *Mastocarpus* sp. Láminas completas (*i.e.*, con un ápice intacto): a) láminas pequeñas (5,0-6,9 cm en longitud), b) láminas medianas (7,0-8,9 cm), y c) láminas grandes (9,0-10,9 cm), considerando seis láminas diferentes para cada estado de desarrollo de las estructuras reproductivas. Segmentos en la lámina: Seg. 1 (basal) a Seg. 5 (apical de las láminas grandes)

Thus, for the two larger blade size-categories, old papillae reach maximum density at a short distance from the apex, and decrease in abundance towards the oldest parts of the blade.

The density of scars differed significantly among segments for all blade size-categories (ANOVA Segment: $F_{(2,10)} = 11.17, P < 0.003$; $F_{(3,15)} = 21.03, P < 0.0001$, $F_{(4,20)} = 23.57, P < 0.0001$, for small, medium and large blades, respectively). Tukey's tests indicated that the density of scars was significantly greater in the basal segment than in the two more distal segments in all size-categories. While scars could be found on small blades, the greatest values were observed in the two most basal segments of medium and large blades, and were scarcely present in the apical segments.

DETACHMENT OF PAPILLAE UNDER LABORATORY CONDITIONS

The detachment of papillae under experimental conditions in the laboratory was evaluated in four blade size-categories. The initial number of papillae only showed a significant effect of blade size-category (ANOVA $F_{(3,6)} = 25.98, P < 0.0008$). Tukey's test indicated that all four means differed from each other. The initial number of papillae was lower in small blades, ranging between 1023 and 1970 in 5 g of fresh blade tissue, and was highest in the large tattered blades, ranging from 2503 to 3061 in 5 g of blades, while medium and large whole blades had intermediate values (1845 to 2531 and 2150 to 2748, respectively). After 24 h of exposure to turbulence in the laboratory, between one and 24 papillae became detached in each replicate of every treatment, representing between 0.05 and 0.93% of the papillae initially present in those replicates. There were no significant differences between the proportion of papillae shed from fronds of different size-categories (ANOVA $F_{(3,6)} = 4.002, P = 0.07$), with an overall average of 0.33 ± 0.06 in 5 g of blade tissue. The interaction term was also non-significant (ANOVA $F_{(6,24)} = 1.857, P = 0.13$), but there were differences among repeats of the experiment (ANOVA $F_{(2,24)} = 4.805, P = 0.03$); Tukey's test indicated that there were significant differences between the first and the third repeat of the experiment, revealing some variation between fronds collected on different days. In all cases the shed papillae had intact pericarps, indicating that spores had not been released. Dissection of these papillae showed that all contained developing carposporophytes with pigmented spores.

DETACHMENT OF BLADES

The force required to detach blades of *Mastocarpus* sp. was 4.0 ± 0.13 N, which was significantly lower (ANOVA $F_{(2,291)} = 176.32, P < 0.001$) than that required to detach blades of *Mazzaella laminarioides* or *Nothogenia fastigiata* (8.5 ± 0.19 and 7.7 ± 0.26 N, respectively). Detachment of blades of *Mastocarpus* sp. always occurred at the narrowest part of the blades, where the fronds emerge from the basal crust. In contrast, for the other two species, blades sometimes broke at more distal parts; as explained above, these values were not included in the analysis.

DRIFTING PAPILLAE

The devices were effective in trapping drifting papillae of *Mastocarpus* sp. Although the number of papillae found was not large, all traps collected between one and five papillae in two days, on each of the three occasions that they were employed, with a mean number of 2.0 ± 0.19 papillae in 78.5 cm^2 entrapment area over the two-day period. This average number of papillae was significantly different from zero ($t = 11.55, n = 3, P = 0.004$). Of the papillae trapped in the collecting devices, all contained at least one developing carposporophyte with normally pigmented spores.

SPORE RELEASE BY DRIFTING BLADES

Blades of *Mastocarpus* sp. are common and easily collected among drift algae. Blades with normal, fresh appearance were abundant. A total of 53 healthy *Mastocarpus* sp. blades were collected. In the laboratory, all of the collected blades released spores with normal appearance and pigmentation.

DISCUSSION

The establishment and expansion of an invading species can be facilitated by the occurrence of multiple dispersing entities (Khou *et al.* 2007). Our results suggest that blades and papillae of *Mastocarpus* sp. could be considered as alternative dispersing structures to spores because they not only detach easily, but they do so when carposporophytes still have spores that could eventually be released. Further studies should determine the ability of such spores to settle and germinate.

Our results indicate that blades of *Mastocarpus* sp. required less force for removal from the rocky surface than two other common, similarly sized, intertidal

seaweeds, namely *Mazzaella laminarioides* and *Nothogenia fastigiata*. The values of detachment force found here were similar to values reported for *Mastocarpus* elsewhere (see Thomsen & Wernberg 2005). The relatively weaker attachment of *Mastocarpus* sp. may result in greater blade loss, with a potentially negative effect on the reproductive potential of the individuals. One possible consequence of blade detachment is a change in the population size structure. Carrington (1990) found that there was no increase in the cross-sectional area of stipes with increasing thallus area in *M. papillatus*. Given that drag on the thallus increased with size, there was greater detachment of larger blades at sites with more wave exposure, which affected the size distribution of blades within this species. Blade detachment due to hydrodynamic forces could also affect reproductive potential, as suggested by Denny & Wethey (2001). Differential detachment of larger blades has also been used to explain differences in abundance between species along a wave exposure gradient. Shaughnessy *et al.* (1996) found that *Mazzaella linearis* was abundant at sites with high wave exposure, while *Mazzaella splendens* was more abundant at less exposed sites. They explain this pattern through morphological and biomechanical differences between the two species. Dudgeon & Johnson (1992) also used mechanical and morphological characteristics in relation to hydrodynamic forces to explain coexistence in mixed stands of *Chondrus crispus* and *Mastocarpus stellatus*. On the other hand, blade detachment can also have a positive effect on the population. In our study, the proportion of blades that broke off from the rocks was not evaluated, but breakage of blades carrying reproductive structures could be frequent, as suggested by the occurrence of *Mastocarpus* sp. in material from the drift line. All of the drifting blades that were tested released spores, indicating that they still had reproductive potential and, therefore, did not represent senescent tissue that had been abscised. Thus, detached and drifting mature blades may act as dispersing entities. Dispersal by entire blades has been suggested before for *M. papillatus* and for other species (Hoffmann 1987, Carrington 1990, Santelices 1990, Norton 1992, Blanchette 1997, Pratt & Johnson 2002).

Not only do mature blades become detached, but mature papillae on the blades also detached when subjected to a moderate turbulence. In the field, observations indicate that some papillae easily fall off simply by touching blades that have been partly desiccated during low tides (R. Otaiza, pers. observ.). The

number of papillae that became detached in laboratory experiments was not high. However, if the daily losses are projected linearly, the accumulated number of shed papillae could be considerable, representing an estimated monthly loss of 14.9% of papillae for the largest blades. Moreover, desiccation and wave action conditions in the field could enhance breakage of peduncles, favoring papillae detachment.

Drifting papillae were also collected in the field. These evaluations were aimed to demonstrate that drifting papillae do occur in the field. The traps were successful in capturing papillae, although their abundance was low. Nevertheless, a linear projection of the average number of papillae collected in the traps would result in over 3000 papillae m⁻² mo⁻¹. If the shedding of papillae represents the elimination of structures that have completed their functional period of hosting carposporophytes, it would be expected to occur after the carpospores were released. Nevertheless, this seems not to be the case, given that all the papillae detached in the laboratory experiment, and also those collected in the traps, contained carposporophytes with well-pigmented spores. Although no germination or spore-viability tests were done, their normal appearance and pigmentation suggested that the spores were viable. In this sense, detachment of papillae carrying apparently viable spores could initially be interpreted as a loss of reproductive potential for individuals, but may result in dispersal if they are capable of releasing spores while drifting away from the parental thallus, or once they come in contact with the substratum. Thus, as with detached whole blades, drifting papillae may also act as important dispersing entities.

In order for blades and papillae to be considered as dispersing structures, there should be a mechanism that produces their detachment at an appropriate moment. Our study shows that the detachment of blades of *Mastocarpus* sp. occurred mainly at the narrow base of the stipe. Other studies show similar results for *M. papillatus* (Carrington 1990, Kitzes & Denny 2005) and for *Mazzaella splendens* and *Mazzaella linearis* (Shaughnessy *et al.* 1996). Some morphological features may facilitate the detachment of blades and papillae at their bases, particularly as they grow and mature. On one hand, allometric growth results in an increase in drag forces acting on the blades, facilitating their detachment. Greater planform area has been associated with greater drag force, so it is expected that dislodgment is facilitated as blades grow (Carrington 1990, Dudgeon & Johnson 1992, Kitzes & Denny 2005). Also, it has been shown that

the presence of structures on the surface of the blades results in increased drag (Carrington 1990). As blades of *Mastocarpus* spp. mature, papillae carrying carposporophytes are produced on the surface as a consequence of sexual reproduction, and as blades grow, the number of papillae increases (West 1972, Polanshek & West 1977, Carrington 1990, Lindstrom *et al.* 2011). On the other hand, as blades grow, their detachment is further facilitated by features that result in the weakening of the attachment points. Cross-sectional area of the stipes may either not increase (*e.g.*, *M. papillatus*; Carrington 1990), or may increase only slightly (*e.g.*, *Mastocarpus stellatus*; Dudgeon & Johnson 1992, Pratt & Johnson 2002), in both cases resulting in a negative allometric relationship with blade area. Hence, the union of the blades to basal structures becomes relatively weaker as blades grow. Moreover, it has been shown for *M. stellatus* that tissue strength decreases with increased stipe cross-sectional area (Dudgeon & Johnson 1992). Therefore, as blades grow and mature, an increase in drag is expected, while the weaker parts of the stipes do not increase proportionately in strength, thus resulting in greater chances of becoming detached. A similar case was described for *Turbinaria ornata* in which fronds become mature, more buoyant and break off, resulting in their dispersal throughout French Polynesia (Stewart 2006).

Detachment of papillae can be explained by a similar mechanism. Papillae on the surface of a blade grow and swell as carposporophytes develop while their basal portion remains a narrow peduncle. This size increase of papillae will increase drag (Carrington 1990) and, in turn, increase the chances of papillae detachment. Thus, detached papillae are expected to be the oldest, mature papillae. This is supported by our results. First, results of the laboratory experiments indicated that the shed papillae were mature papillae containing spores. Second, the pattern of distribution of new and old papillae, and scars, differed along the blades. While new papillae were more abundant on the apical, younger segments, and mature papillae were more abundant on the middle segments, scars of shed papillae were more abundant at the base of the blades (Fig. 2). Also, scars were more abundant on large and mid-sized blades than on the smaller blades (Fig. 2). These results show a distinctive release pattern, where shed papillae were the oldest ones, occurring on the oldest parts of the blades.

In this species, the chances of blades and papillae becoming detached from the parental thallus and drifting away increase with the size of the structure, and

consequently, the maturity of the carposporophytes they carry. Also, both papillae and blades with papillae can release spores and, thus, may have the potential to produce new individuals. Therefore, both blades and papillae can be considered as propagules, representing dispersing structures in addition to tetraspores and carpospores. *Mastocarpus* sp. is a proposed invader in Chile (Castilla *et al.* 2005). While the moment and locality of arrival or the vector transporting this species to Chile are not known with precision (Castilla *et al.* 2005), we propose that having additional dispersing entities alternative to spores may have favored its establishment and subsequent expansion.

Dispersing entities that are structurally very different from spores may represent advantages for dispersal in several ways. One advantage is that dispersal trajectories and arrival sites of these structures may be different from those of spores. Blades and papillae differ from spores in their buoyancy, so it is expected that once detached, they will sink to the subtidal zone and be subject to currents there. Spores, on the other hand, will most likely remain suspended in the water column (Gaylord *et al.* 2002, Opazo & Otaiza 2007) and be transported by more superficial water currents. Hence, the release or detachment of different types of structures may result in the transport of propagules to, and the colonization of, different habitats. Furthermore, blades or papillae may also release spores while drifting. A second advantage of additional dispersing entities is that these multicellular structures may undergo considerable dispersal distances before they release spores. Extreme cases include the long distance, trans-oceanic transport reported for some kelps and fucoids (Hobday 2000, Thiel 2003, Macaya *et al.* 2005, Thiel & Gutow 2005, Stewart 2006, McKenzie & Bellgrove 2008), but local transport by nearshore currents may be a much more frequent means of dispersal. In contrast, the short viability period of spores released by the attached parental thalli limits the distance that they can effectively disperse (Norton 1992, Bobadilla & Santelices 2004, 2005). A third advantage is that spores inside these multicellular dispersing structures should have reduced mortality while drifting in the water column given that they are protected and nourished by the adult tissue (Hommersand & Fredericq 1990). Unicellular entities, such as spores, released singly or in gelatinous matrices are at high mortality risk in the water column and on the substrata once they settle (Santelices 1990, Amsler *et al.* 1992). Finally, upon arrival to the shore, a multicellular entity may release a mass of spores onto a hard substratum.

Groups of spores in close proximity may coalesce and produce a single new individual (Santelices *et al.* 1999). As a consequence, an individual growing from a group of spores will attain a larger size more rapidly than one that grows from a single spore. This should reduce mortality related to small size, particularly at the germling stage (Santelices & Alvarado 2008).

Our results show that blades and papillae have features that facilitate their breakage from the parental thallus as they grow and mature. This does not represent abscission of empty or senescent structures. Instead, these structures may actually be important in facilitating the establishment of new individuals at distances and places that may be different from what is achieved by spores. Hence, they could be considered as alternative dispersing entities (Carrington 1990, Blanchette 1997, Pratt & Johnson 2002). To better understand the population dynamics and range expansion of *Mastocarpus* sp. in Chile, it is necessary to determine the success and relative contribution of each of these dispersing entities to the population.

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