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Utilization of holdfast fragments for vegetative propagation of *Macrocystis integrifolia* in Atacama, Northern Chile

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Abstract We attempted to propagate *Macrocystis integrifolia* (giant kelp) by fragmentation and regeneration of holdfasts, which consist of creeping stolons with lateral haptera. Stolons from a natural kelp population in Bahia Chasco (Atacama, North Chile) were cut into fragments, each containing one or more lateral stipe and frond initials. Fragments were attached to longlines with elastic bands. We used two additional types of inoculants as controls: (1) natural recruits from the local parent population and (2) laboratory-cultured young sporophytes. Length, reproductive phenology, and mortality were determined monthly. Our results confirmed the feasibility of *M. integrifolia* propagation by reattachment of stolon fragments, and we obtained up to eight new individuals from one parent holdfast. Individuals from holdfast regeneration formed sporangial sori 3 months earlier than control specimens from recruits and laboratory culture, while all three types gave similar values for mortality. Holdfast morphology of regenerates differed strongly depending on their origin: stolon fragments continued growth and developed new haptera except in the cut surfaces, natural recruits formed typical *M. integrifolia* holdfast morphs, and sporophytes originating from laboratory culture produced minor stolons with many haptera. Implications of our results on ecology, repopulation, and aquaculture are discussed.

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Introduction

Two processes are known for multiplication of macroalgal biomass in nature: (1) reproduction, which uses spores and gametes to produce new thalli, and (2) vegetative propagation, by which somatic tissue of a parent thallus gives rise to new individuals. Reproduction, typically combined with sexual recombination, is a universal process, enhancing genetic diversity, dispersal, and natural population dynamics (Santelices 1990). Thallus fragmentation has been described in red and green seaweeds, mostly as a secondary mechanism for population dispersion and preservation (Pacheco-Ruiz et al. 2005; Smith and Walters 1999). In some cases, thallus fragmentation has been applied in mariculture and population management as a strategy offering faster and less expensive results than traditional spore-based methods (Correa et al. 1999; Gómez and Westermeier 1991; Macchiavello et al. 2003; Westermeier et al. 1993, 2012a).

The life history of *Macrocystis* includes sexuality: microscopic gametophytes produce diploid zygotes by sexual fusion. These grow up to the dominating kelp sporophyte, which forms meiospores on sporophylls to complete the cycle (North 1971).

According to Westermeier et al. (2012b), the *Macrocystis integrifolia* beds of Bahia Chasco, Atacama are quasi-perennial. Adult specimens release their meiospores in spring to summer. Subsequently, thalli erode and partly die off. In parallel, new thalli emerge from the perennial holdfast. Spore germination, gametophyte development, gametogenesis, and fertilization presumably occur during autumn

to winter, since juvenile thalli appear in spring and quickly develop towards summer. There are no literature reports on vegetative propagation in *Macrocystis*, although some authors discuss this process as a main mechanism for population stability (Lobban 1978; Graham et al. 2007). Some studies on kelp repopulation projects and mariculture trials showed that whole thalli could be successfully transplanted to rocks or longlines (Correa et al. 2006; Graham 2008; Westermeier et al. 2006, 2011), reflecting high capacity of kelp reattachment.

M. integrifolia has a stoloniferous holdfast (comparable to higher plant rhizomes or stolons) with lateral extensions called haptera (Hoffmann and Santelices 1997). This creeping character, the possible fusion of neighboring holdfasts, and the high capacity of natural repopulation in nature (Lobban 1978; Graham et al. 2007; Westermeier, unpublished) suggested the possibility of vegetative propagation of *M. integrifolia* through holdfast fragmentation.

Our experimental material in Bahia Chasco corresponds to *M. integrifolia* Bory as described and illustrated by Hoffmann and Santelices (1997). A stoloniferous holdfast is the key character that separates *M. integrifolia* from *Macrocystis pyrifera*. These two taxa are geographically separated along the coast of Chile but occur sympatrically in Peru and North America. Based on their interfertility (Westermeier et al. 2007) and supported by morphological plasticity and molecular data, Demes et al. (2009) and Macaya and Zuccarello (2010) suggested their conspecificity under the name *M. pyrifera*. Since our present study focuses on the *M. integrifolia*-specific holdfast character and there is presently no agreement on its subspecific status, we prefer here to maintain the taxon epithet *M. integrifolia*.

Materials and methods

Our study site was Bahia Chasco (27°40′ S, 70°59′ W; Atacama region, North Chile), where a large population of *M. integrifolia* occurs. Stolons of 20–40 cm in length were cut into small pieces of approximately 3–6 cm, each holding one to two stipes and several fronds (Fig. 1a). These were fixed with elastic bands onto longlines of 5 m, at a density of 3 inoculants m^{-1} (Fig. 1b). As control groups, we inoculated onto parallel longlines (1) juvenile kelp recruits from the natural populations of Bahia Chasco (Fig. 1c) and (2) young sporophytes from laboratory culture. These individuals were produced from spores previously collected at Bahia Chasco and grown for ≈ 30 weeks to explantation size in our Universidad Austral de Chile Seaweed Laboratory at Puerto Montt (Fig. 1d).

The age of recruits and holdfast fragments taken from the natural habitat could not be determined; 30 randomly selected replicates per pretreatment were explanted and exposed in the sea from June 2011 to March 2012. Supporting structures were exposed at 7–8 m depth and were similar to *Macrocystis*-, *Lessonia*-, and *Gigartina* mariculture installations described by Westermeier et al. (2006, 2012a) (Fig. 1e). The lines were hauled in for measurements of growth, reproductive phenology (percent reproductive individuals), and mortality at monthly intervals.

Results

Upon exposure to the sea, inoculants from all three pretreatments formed new haptera to fix themselves on the longlines (Fig. 1f–h). They continued to grow and became fertile within a few months (Fig. 2). Holdfast fragments grew not

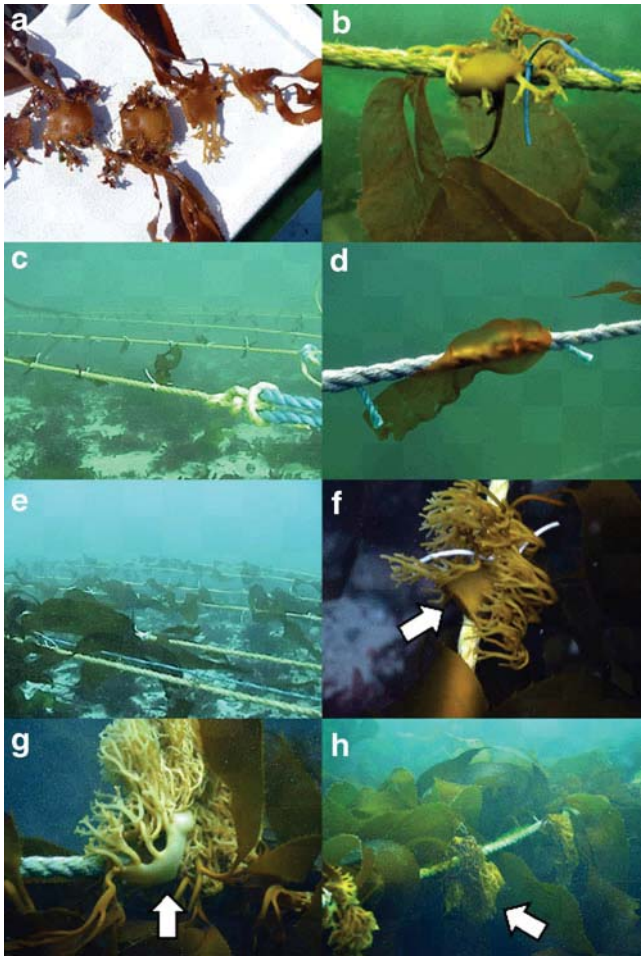

Fig. 1 Vegetative propagation and reattachment of *M. integrifolia* on longlines. **a** Older *M. integrifolia* holdfast cut in small pieces, conserv ing some stipes and fronds; **b** holdfast fragments; **c** recruits from Bahia Chasco; **d** young sporophytes from culture, inoculated on longlines; **e** panoramic view of *M. integrifolia* mariculture installations (sensu Westermeier et al. 2006); **f** morphology of holdfast fragments after 9 months of sea culture, indicating scar of fragmentation cuts (*arrow*); and **g**, **h** recruits and culture sporophytes, respectively, after 9 months at sea, indicating holdfast morphology

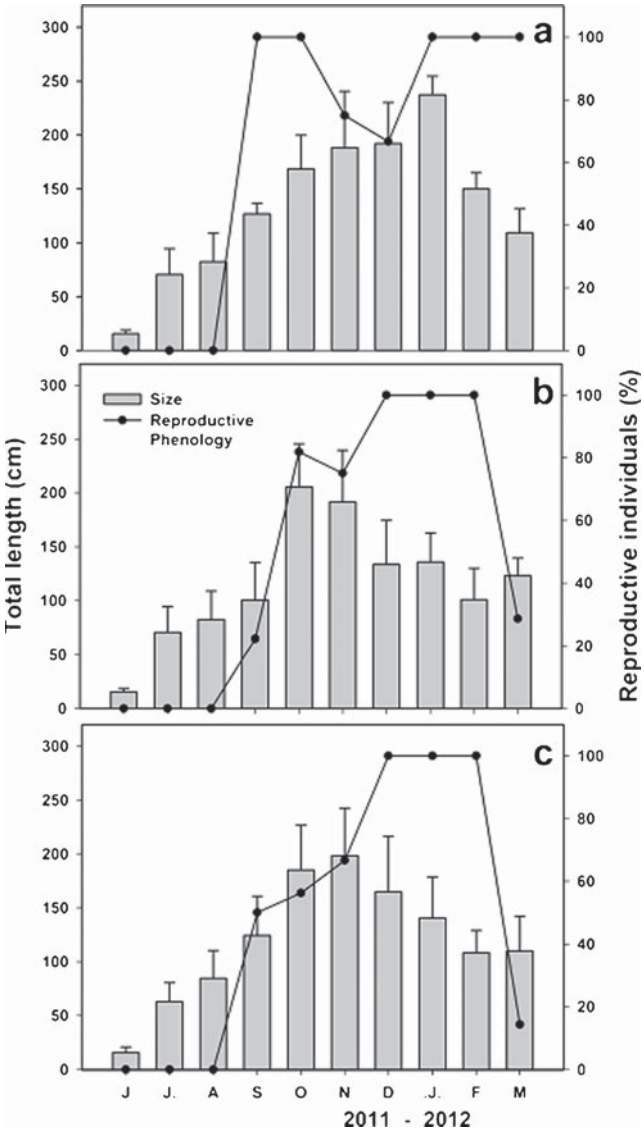

Fig. 2 Growth (*bars*) and reproductive phenology (*lines*) of *M. integrifolia*. **a** Holdfast fragments, **b** recruits, and **c** sporophytes from culture

only more slowly but also constantly, reaching maximum values in January (ca. 230 cm), and decreased in length toward late summer (Fig. 2a). Growth of fronds was similar in recruits and culture sporophytes, reaching maximum sizes of ca. 200 cm in 4–5 months, followed by a loss in frond size to ca. 100 cm in subsequent summer months (Fig. 2b, c).

Reproductive phenology, instead, showed different trends. In holdfast regenerates, 100 % of individuals reached reproductive maturity 3 months earlier than recruits and culture sporophytes (Fig. 2). Nevertheless, a significant proportion of regenerates from all three pretreatments were fertile during the entire experimental period. Regenerates from all three treatments showed severe apical erosion in summer months and loss of reproductive tissue after sporulation, resulting in depression of frond lengths.

Maximum mortality was recorded for holdfast regenerates (33.3 %), followed by fronds originating from culture (26.7 %) and recruit transplants (13.3 %). The "mortality" recorded in our study reflects basically failure of holdfast attachment, leading to loss of individuals from the longline. All successfully attached regenerates remained on their place on the longlines and grew healthily.

We observed differences in holdfast morphology. Regenerates from holdfast fragments developed new haptera to attach to the longline, but the scars from the original cutting remained inactive (Fig. 1f). In contrast, recruitment individuals increased rapidly in size and formed typical stoloniferous *M. integrifolia* holdfast morphs (Fig. 1g). Individuals from cultured inoculants produced prolific haptera with reduced stolon formation (Fig. 1h).

Discussion

Growth of *M. integrifolia* on longlines in our present study was similar to that reported for *Macrocystis* in Northern and Southern Chile (Westermeier et al. 2012b; Westermeier and Möller 1990). Maximum frond length was found in spring, followed by a decrease in summer. In Northern Chile, specifically in Bahía Chasco, specimens can reach 5 m in length in spring, which subsequently drops sharply (0.75 to 1 m in summer to autumn, Westermeier et al. 2012b). This trend reflects environmental changes and a natural sequence of population cycle (recruitment, growth, reproduction—with a massive spore release—and death; Lobban 1978). Furthermore, our study showed that regenerating holdfast fragments grew more slowly than normal recruits, but they reached reproductive state more rapidly. This indicates that thallus age is an important criterion for growth and reproduction, perhaps reflecting activity of hormonal factors.

Our study indicated that holdfast morphology of *M. integrifolia* in Chile is subject to considerable plasticity, with substratum, water current, and depth exposure as important factors. Nevertheless, genetic differences to *M. pyrifera* holdfast expression clearly exist, as has been shown in hybridization experiments between Chilean *M. pyrifera* and *M. integrifolia* (Westermeier et al. 2007; Westermeier, unpublished).

Our study showed that it is possible to use juvenile natural recruits as well as holdfast fragments for transplantation. Both reattach easily to any nontoxic substratum, grow normally, and reach reproductive maturity. The procedures developed in our project provide an important basis for economic activities of local fishermen in Bahia Chasco and other localities where *M. integrifolia* is exploited. The holdfast of one adult specimen can be cut into eight or more fragments and fixed to selected new substrata where they will form new individuals. Also, the high reproductive

potential of transplanted *M. integrifolia* specimens can be used to colonize new or to recolonize areas devastated by overharvesting. The methods described here are inexpensive and easy and can be used for repopulation purposes as well as small-scale mariculture programs.

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