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# Germplasm banking of the giant kelp: Our biological insurance in a changing environment

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ABSTRACT

Genetic diversity is being lost at a fast pace — seaweeds are no exception. The giant kelp, *Macrocystis pyrifera*, forms vast underwater forests in both hemispheres and is a key species for ecosystem functioning. But this species is also a commodity product. *M. pyrifera* is harvested for its chemical compounds (*e.g.* alginates) and for feedstock (*e.g.* abalone). In the past 5 years, some companies tried new farming techniques to boost biomass production for biofuel conversion. But the lack of sustainable management can lead to genetic erosion and degradation of livelihoods. Often, the natural genetic populations are not described, and we may be losing what is yet to be found. Aiming to alert and prevent this situation, we developed a germplasm bank based on the genetic diversity of *M. pyrifera* from Chile. We preserved female and male gametophytes in separate, from 3 genetic populations in low light, at 10 °C, in Provasoli media but without cryoprotective agents. After 5 years in cold storage, we show for the first time gametophyte viability up to 89% and viability differences between genetic populations. We discuss the benefits of this germplasm bank considering sustainability of seaweed production, food security pressures, and climate change.



## 1. Introduction

Macroalgae are one of the most biologically important elements of worldwide marine systems and of major importance for ecosystem functioning, aquaculture, and downstream industries [1]. Seaweeds have long been considered a valuable biological resource in Asia [2,3]. However, only recently the Western society became re-aware of seaweeds as a resource instead of a nuisance. The main drivers for this recent interest are well known — fuel security, food security and aquaculture sustainability [2,4–6]. In the past 5 years Western governments and biotechnology companies have invested millions in research projects to develop seaweed aquaculture (Europe: Seaweedstar, Seaweedtech, Seaweedbreed, EnAlgae, MABFUEL, AT-SEA; Chile: BalBiofuels; Canada: Acadian Seaplants, Limited) and boost the biomass production mainly for biofuel purposes. Small farming companies emerged out of the blue targeting niche markets among educated middle-class consumers influenced by modern chefs promoting sustainable healthy cuisine [7]. A new generation of scientists insisted in the need to use seaweeds for bioremediation purposes and a more sustainable integrated aquaculture industry [8–14].

Despite the intention to grow big, the Western industry of seaweed biomass production is in its infancy or arguably non-existent. Moreover, none of the projects so far addressed the need to develop a sustainable harvesting alongside a sustainable domestication program, and little is known about genetic diversity among commercial seaweed species [15]. In Asia the industry urgency and constant demand for improved production traits (*e.g.* higher growth rates) resulted in inefficient breeding programs, seaweed genetic diversity loss and the introduction of alien genotypes to other wild species [16–20]. Some reports refer to a production decline after 3 years of steady growth, this is usually a consequence of the outbreak of pathogens, increased herbivory, and epiphytes [21,22]. Climate change and extreme weather events also affect seaweed crops — on the 11th of March of 2011, the aquaculture of *Undaria pinnatifida* along the Sanriku Coast was almost completely destroyed by the tsunami that hit Japan [23]. In 2009 and 2010, the production of *Porphyra yezoensis* crashed in Jiangsu Province, China as a consequence of global warming [24].

Worldwide there are several examples of loss of genetic diversity of farmed seaweed species (Table 1), mostly of red seaweeds but also of kelps. The seaweed aquaculture industry relies in the serial subculture of restricted germplasm banks. Ye et al. [6] found that the *Saccharina* cultivars that are currently farmed in China, have a low genetic diversity because they were derived from limited crossbreeds from the hybridization of *Saccharina japonica* and *Laminaria longissima*; suffered from a restricted germplasm base; and continuous selfing. According to these authors to increase the genetic diversity, improve quality and yield we need to go back to the wild-type. More recently the word re-wild was introduced by Andersen et al. [25] in a controversial review suggesting the reintroduction of wild-type genes from ancient

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**Table 1**
Examples of genetic diversity loss reported in farmed seaweeds worldwide.

| Group | Species | Region | Country | Reference |
|---|---|---|---|---|
| Red | *Porphyra tenera* | Asia | Japan | [18] |
| | *Gigartina skottsbergii* | South America | Chile | [16] |
| | *Gracilaria chilensis* | | | [17] |
| | *Eucheuma* and *Kappaphycus* | Africa | Zanzibar | [29] |
| Brown | *Saccharina japonica* | Asia | China | [6,30–34] |
| | *Undaria pinnatifida* | | | [35] |

plant crops into organically farmed plants. In this scenario, new breeding techniques would be used to re-wild organic crops and improve production yields. This concept has been suggested before [26,27] but the sound bite word — re-wild, was quickly caught by world press (*e.g.* New York Times; [28]) and disseminated in social media.

If in the western world we want to keep a healthy environment and at the same time develop a resilient and sustainable industry, we need to adopt pro-active production management strategies learned from the vast research efforts developed for aquaculture species, terrestrial plant and livestock. And the first step is to preserve the wild-type, and thus the genetic diversity, which like species diversity can provide biological insurance against environmental change both locally and globally and from pressures from the industry.

Worldwide there has been a consistent effort to create seed banks for many terrestrial plant species [26] and collections of microalgae strains of economic importance. For microalgae there is now a reasonable number of institutions that own microalgae cryopreservation banks (*e.g.* Europe: Culture Collection of Algae and Protozoa (CCAP) (UK); Sammlung von Algenku Huren Göttingen (SAG) (Germany); North America — The Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP) (USA), The Culture Collection of Algae at the University of Texas at Austin (UTEX) (USA), Asia — National Institute for Environmental Studies (NIES) (Japan)). But there is so far not a single example of a dedicated macroalgae collection and long-term storage of seaweed species and strains of both economic and ecological interest. The main reason for this is probably the difficulties and limited success of freezing macroalgae material using cryopreservation techniques (storage of viable cells at low temperatures) for long periods (*e.g.* over 100 days) [36,37].

The giant kelp, *Macrocystis pyrifera*, can form extensive underwater forests, it is the largest seaweed, and one of the fastest growing photosynthetic organisms found on earth [38]. It also happens to be the food of choice of abalones. In Chile, the farming industry of abalone is growing at a fast pace, and with it, the harvesting of the giant kelp [39]. Recent studies reported the erosion of some natural kelp forests as a consequence of harvesting [40].

Aiming to preserve genetic diversity of the giant kelp, *M. pyrifera*, a germplasm bank framework for individual female and male gametophytes from three different genetic populations in Chile was developed. This framework involves three fundamental steps: 1) genetic screening of the wild populations; 2) germination, selection and individualization of female and male gametophytes from the different populations; and 3) implementation of a reliable cold storage preservation technique.

We chose to develop a cold storage technique that keeps the gametophyte cells in suspended state, and is inexpensive; technically simple to implement, and gives a high degree of viable cells after long-term storage (years). This technique is an alternative to the serial subculture of actively growing cells, which is a common practice in culture collections but is costly, time-consuming and requires skilled technical staff [36,41,42]. To prove our protocol was successful, we evaluated the morphological characteristics and viability success of preserved female and male gametophytes after 5, 4, and 3 years from 3 genetic populations of *M. pyrifera* from Chile. We addressed the following scientific questions: Do the gametophytes show morphological and viability changes as a consequence of 1) storage duration; 2) different sexes; 3) different genetic populations? What are the benefits of having a germplasm bank based on genetic screening?

## 2. Material and methods

### 2.1. Germplasm collection

Fertile sporophytes (20 to 30 individuals) were collected by scuba diving at 9 localities along the Chilean coast in three consecutive years — 2010, 2011 and 2012 (Fig. 1). Sampling points were selected in order to cover the complete distribution of the species in the Chilean coast. Each individual plant was tagged and transported in mesh bags (2 to 3 plants/bag) to the laboratory within 4 h of sampling. A total of 2 to 4 sporophylls were selected per plant, and then washed with fresh water, dried with paper towel and covered with another paper towel and aluminum foil. This package was kept overnight at 10 °C. On the following morning, the mature sorus were individualized with a punch hole and a total of 10 pieces (circles of 3 cm diameter) were introduced in 250 mL Erlenmeyer flasks with autoclaved and filtered (0.22 μm membrane pore, Millipore) natural seawater. Sporulation usually occurs after 30 min. For all samples from every population and year spores were counted and a total of 20,000 spores/mL were seeded in Petri dishes with filtered seawater enriched with Provasoli culture medium [43]. Cultures were transferred to 10 °C, 16L:8D photoperiod and irradiance of 20 μmol photons $m^{-2}$ $s^{-1}$. When female and male gametophytes were distinguishable, isolation of gametophytes separated by sex was performed using a glass Pasteur pipette and transferred to a 96-well multi-plate — one gametophyte per well. For each plate only 48 wells were used, that is 24 for females and the remaining 24 for males. Once completed, each multiplate was placed at 10 °C with a light intensity of 5 μmol photons $m^{-2}$ $s^{-1}$ to avoid further development.

### 2.2. Genetic population determination

Blade tissue from each individual was excised, washed with fresh water and immediately placed into plastic bag with silica gel crystal. Total genomic DNA was isolated using protocol proposed by Wattier et al. [44] with minor modifications: before adding lysis buffer the dried frond was fine powdered in a mini beadbeater in tubes with stainless steel beads and PVP (polyvinylpyrrolidone), instead of milling with liquid nitrogen as suggested by Wattier et al. [44]. Ten microsatellite loci were selected from Alberto et al. [45] (Mp-BC-4; Mp-BC-13; Mp-BC-16; Mp-BC-25; Mpy-7: Mpy-9; Mpy-11; Mpy-14; Mpy-17 and Mpy-19). PCR reactions were carried out according to [45] with minor changes to the concentrations of reagents in the master mix. PCR products were analyzed on an ABI PRISM 310 automatic sequencer (Perkin Elmer, Waltham, MA, USA) using the 500 LIZ standard (Applied Biosystems, Foster City, CA, USA). Raw allele sizes were scored with GeneMarker software version 1.95 (Softgenetics) and binned using FlexiBin [46]. Molecular data analysis was according to Camus et al. (in press).

### 2.3. Gametophytes morphology characterization

In February 2015, we observed five plates per population (North, Centre and South), from 9 locations, of each year (2010, 2011 and 2012) in a total of 45 plates and 2160 wells (Fig. 1). Each isolated gametophyte, from each population, collection year and sex, was characterized according to: 1) morphology — presence of typical cell structures (plastids, membranes); pigmentation — presence of brown pigments; and degree of contamination (Fig. 2). The morphological characteristics and pigmentation degree were compared to a control of gametophytes which germinated from spores of fertile sporophytes collected by scuba diving from Metri in December 2014 and kept in culture until March 2015 (spore release and gametophyte culture was done following the