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Using sporeless sporophytes as a next step towards upscaling offshore kelp cultivation

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Summary

The feasibility, benefits, risks, and consequences of using sporeless sporophytes to aid the upscaling of kelp cultivation in Europe and North America, as well as the barriers that currently exist and how these may be overcome, are reviewed here. Taking environmental, industrial, and regulatory factors into account, we will discuss the use of domesticated sporeless sporophytes as an asset to facilitate upscaling kelp cultivation without potentially impacting wild genetic diversity.

Keywords

Kelp, cultivation, sporeless sporophytes, natural variation, macroalgae, regulations

Declarations

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Introduction

The human population is projected to hit 9.7 billion by 2050 (UN 2022), which the current state of global food security will not be able to sustain. With the additional threat of climate change, there is an urgent need for a worldwide transition from energy-, freshwater-, and arable land-intensive crops to low-carbon crops like farmed seaweed to provide, for example, food, feedstocks for animal feeds, biofuels and bioplastics. Seaweed farmed as a crop has the potential to facilitate the sustainable, economic, and ecological transition to a circular economy in food and energy production (Aiking and de Boer 2020; van den Burg et al. 2021). Seaweeds require little space to grow and are rich in essential nutrients, minerals, carbohydrates and amino acids which can be used as functional foods or feedstocks for further processing. However, seaweed cultivation is in its early stages in Europe and North America, with many remaining scientific and technological challenges to overcome, presenting opportunities for innovation to increase the size and scale of seaweed farming (Campbell et al. 2019; Kim et al. 2019). Here, we focus on the opportunity to selectively breed economically important kelp species whilst protecting wild genetic diversity and addressing regulatory concerns. Creation of sporeless sporophytes of selectively bred kelps is deemed a key enabling technology for domesticating kelp and upscaling farming (Valero et al. 2017; Goecke et al. 2020). We have chosen the term “sporeless” to indicate infertile, sterile individuals not capable of reproducing and have chosen to focus on kelp seaweed species (Laminariales) as they have high potential for larger scale cultivation. We define sporeless as the inability of sporophytes to produce reproductive sorus tissues with meiospores or produce normal meiospores that can develop into fertile gametophytes. Whilst not anatomically correct, with lay audiences we equate this to and sometimes use the term “seedless” which is generally understood. To our knowledge, this is the first publication to specify in detail different means for producing infertile kelp strains, despite a previous publication (Li et al. 2016) which suggests that seedless kelp have been produced. A close reading reveals that the strain Dongfang #7 is routinely reproduced from itself and the text makes no further mention of seedless kelp.

Kelp cultivation

For seaweed cultivation to reach its socio-economic and market potential, yields need to be increased, for which research and innovation in selective breeding (Charrier et al. 2015; Liu et al. 2017) and biorefinery processes (Patyshakuliyeva et al. 2019; Torres et al. 2019) are urgently needed (Buschmann et al. 2017; Bak et al. 2018). In 2020, 36.17 million tonnes of algae were produced globally, of which 97% was cultivated seaweed (FAO 2022). The vast majority of cultivated seaweed is produced in East and Southeast Asia, whilst the majority of seaweed produced in Europe is still wild-harvested. To meet predicted future demands for seaweed and seaweed-derived products for human consumption, some of which may exceed wild-harvesting limits, scaling up ocean farming will be necessary. However, scaling up seaweed cultivation poses logistical challenges, requiring profitable practices whilst minimising any negative impacts on marine resources (Loureiro et al. 2015; Grebe et al. 2019; Visch et al. 2020). Selective breeding techniques capable of cultivating superior strains will enable the quality and quantity of cultivated seaweed necessary to be competitive with other similar products, for example, high-performing kelp strains from Asia, other seafood-based products and, more broadly in the future, with terrestrial crops which have been selectively bred for hundreds of years.

Selective breeding

Presently, the majority of seaweed farms in Europe and North America are growing kelp species cultivated from wild spores or "seed" collected from local kelp blades. This is the traditional approach and, if enough blades are sampled, it ensures that farmed kelp resembles the local wild kelp, thereby reducing the risk of any potential genetic impacts that could be caused by farmed and wild kelp interbreeding in the environment (Goecke et al. 2020). This is often the approach required by regulating marine resource managers in the U.S.A. who are responsible for protecting wild kelp (e.g., Maine requires stock originating in Maine waters; Maine Legislature 2017; and Alaska requires gathering stock from within 50 km of the seaweed farm; Gruenthal and Habicht 2022; State of Alaska 2022). Further, regulators have codified concerns about alien and 'locally absent' aquacultured species introductions in European environmental legislation, e.g., Council Regulation EC No. 708/2007 of 11 June 2007 (Barbier et al. 2019), and U.S. regulators have stipulated where and how many parents must contribute when breeding seaweed (State of Alaska 2022). However, restricting cultivation to local varieties inhibits the profitable upscaling of kelp cultivation to the degree achieved with selectively improved kelp as seen in Asia (Hu et al. 2021; Hwang et al. 2022).

Whilst selective breeding on a research scale (e.g., one- meter plots per MARINER projects; Umanzor et al. 2021; Li et al. 2022) has not posed a risk to wild kelp, it is essential to be proactive about ensuring that larger selectively-bred kelp farms of the future do not overwhelm and irreversibly genetically alter wild kelp populations. A well-accepted method of practically eliminating this risk in the cultivation of higher (terrestrial) plants is the use of sporeless or seedless individuals (e.g., Yamamoto 2014; Akkurt et al. 2019; Wijesinghe et al. 2020). These same or similar methods can be applied to kelp to induce reproductive sterility (Goecke et al. 2020). Using a method that is already widely used in other terrestrial and aquaculture crops suggests that it is likely to be accepted by regulators and consumers purchasing the resulting product (Mather and Fanning 2019), as there is already a familiarity and understanding of the concept. There are at least two ways of producing sporeless individuals: by producing polyploids or by selecting naturally occurring sporeless mutants from the wild population and breeding them such that sterility becomes genetically dominant.

Alternatively, other approaches to reduce or prevent reproduction between farmed and wild populations could include selecting for delayed reproduction in farmed strains to occur only after the normal harvest season. Another approach would be to locate farms far from natural kelp beds (offshore) or outside of their natural distribution in regions where kelp may be thermally tolerant in the winter but propagules cannot survive the summer and thus cannot complete their lifecycle. These latter alternatives are not the topic of this paper however, and we consider using sporeless individuals as the most promising route towards upscaling sustainable kelp cultivation for reasons further described below.

Precedent for infertility in agri- and aquaculture

Producing infertile crops through polyploidy has been successfully achieved for years both in agri- and aquaculture and has led to improved yield, advancements in genetic manipulation and increased biosecurity. For example, commercial oyster hatcheries commonly breed triploid oysters, typically the product of diploid females crossed with tetraploid males (Guo et al. 1996). Triploid oysters have two advantages over normal diploid lines; they exhibit faster growth rates, reach market size sooner and thereby reduce cultivation time, and they retain their meat quality through the summer months due to lack of reproduction (Wadsworth et al. 2019). This also prevents selected oyster varieties from genetically mixing with wild oyster populations, thus, the use of infertile strains in crop production may serve both breeders and the environment. A relevant example pertinent to resource management is the use of triploid grass carp for aquatic vegetation management. Allen and Wattendorf (1987) illustrates the verification and certification processes in place for regulatory approval and use of triploid carp which would otherwise be invasive.

In higher plants such as watermelon, the goal of making triploid varieties is to avoid seed set in the produced fruits. A homozygous diploid pollinator fertilises a homozygous tetraploid mother plant. The hybrid formed is triploid and will undergo an unbalanced meiosis at flowering which causes both male and female sterility. Triploid varieties in watermelon have been grown since the 1990s and have also been seen to have beneficial traits such as a thicker rind and firmer flesh (Wijesinghe et al. 2020).

Lifecycle of kelps

The kelp life cycle differs from that of higher plants in that the microscopic gametophytic life stage of kelp is physically independent from the macroscopic sporophytic stage. This adaptation can be very useful when domesticating and cultivating kelp since gametophytes can be clonally propagated and maintained as parental material in labs or nurseries for years. If desirable traits can be identified and selectively bred, clonal lines of a favoured genotype can be produced and maintained in a vegetative state to be retrieved and fertilised as required (Ebbing et al. 2021). Selective breeding can be used to produce higher yields or any other desirable trait beneficial for farming and the end product, as it has over hundreds of years for a large variety of crops, including those specifically bred for organic standards (Lammerts van Bueren et al. 2011). Results from the MARINER program have demonstrated improvements in selectively bred varieties of *Saccharina latissima* that amount to twice the typical commercial yields and up to 28 kg m^{-1} wet weight and 4 kg m^{-1} dry weight (Li et al. 2022). The ability to safely cultivate selectively bred kelp without genetic risk to the environment would transform and expand the kelp aquaculture industry in Europe and North America by increasing its efficiency and reliability.

This paper examines the feasibility of the production of sporeless sporophytes and the benefits, risks and consequences thereof, as well as the barriers that currently exist and how these may be overcome. Taking environmental, industrial, and regulatory factors into account, we will discuss the use of sporeless sporophytes as an asset to facilitate upscaling kelp cultivation in Europe and North America and potentially other seaweed farming regions of the world.

Sporeless kelp sporophytes

There is similar potential to achieve infertility in kelp through the production of triploid sporophytes which cannot successfully undergo meiosis to produce viable haploid gametophytes. The independent life stages of the kelp gametophyte and sporophyte mean that it is possible to cross one haploid and one diploid gametophyte to form a triploid sporophyte.

Homozygous diploid kelp gametophytes could be produced chemically by applying chromosome doubling agents to a haploid gametophyte, such as oryzalin or colchicine. This has yet to be proven on macroalgae, but has been performed successfully on microalgae (Bafort et al. 2023) and higher plants (e.g., Chauvin et al. 2003). Of the two, oryzalin has been shown to be the more efficient chromosome doubling agent and less likely to cause development of chimaeras than colchicine (Sree Ramulu et al. 1991; Bouvier et al. 1994; Chauvin et al. 2003). Diploid gametophytes can also occur naturally, as found in *Alaria* (Feller-Demalsy and Demalsy 1974), and it has been suggested that higher environmental stressors increase the occurrence of diploid gametophytes in *Laminaria digitata* (Oppliger et al. 2014). It would require further investigation to determine whether these conditions could be artificially replicated in kelp to induce higher numbers of diploid gametophytes and what number could be obtained in this way. Diploid gametophytes can then be identified and isolated via cell sorting flow cytometry as demonstrated with kelp meiospores (Augyte et al. 2020).

The diploid gametophytes can be propagated and maintained as separately contained male and female gametophyte monoclonal cultures in a vegetative state (Lüning and Neushul 1978). Thus, each culture container propagates a single genotype. Gametophytes from these cultures can be used for fertilisation and production of triploid sporophytes by cross-breeding select genotypes of the male and female gametophyte. Such cultures can be maintained for years (Barrento et al. 2016), produce genetically consistent sporophytes, and breeding would no longer be subject to natural seasonal limitations. Desired phenotypes can also be selectively bred and kept in the culture. This process can be scaled up to store large numbers of gametophytes to supply genetically controlled and bio-secure breeding material to commercial kelp growers. Gametophytes can also be stored long term by cryopreservation (Visch et al. 2019). It has yet to be seen whether polyploidy in kelp would yield the additional advantages sometimes found in other triploid species such as increased individual cell size (Orr-Weaver 2015) or heterozygosity and heterosis or hybrid vigour (Sattler et al. 2016).

Another route to producing sporeless varieties could be selecting wild individuals with naturally occurring recessive sporeless alleles and breeding complementary males and females so that the sporeless allele is homozygous and dominant. For example, as meiosis is a crucial step for spore production, natural mutations found on meiosis checkpoint genes could result in sporophytes that will not produce meiospores and thus be sporeless. Likely candidate genes for controlling meiosis and fertility, OUROBOROS and SAMSARA, have been identified in the model brown alga *Ectocarpus*, and are responsible for conversion of the sporophyte generation into a gametophyte (Arun et al. 2019). Such a cross can be achieved within one generation using gene sequencing to identify appropriate mutations on such genes. Such mutations should not be difficult to find since whole genome sequencing has already been completed in kelp species such as *Saccharina latissima* (Mao et al. 2020; Huang et al. 2023), *Macrocystis pyrifera* (Molano et al. 2022; Diesel et al. 2023; Gonzalez et al. 2023), *Alaria* (Bringloe et al. 2021) and *Undaria pinnatifida* (Shan et al. 2020). A future dedicated macroalgae germplasm bank of a large diversity of species would greatly aid in screening and maintaining naturally sporeless varieties (Wade et al. 2020; Molano et al. 2022).

Potential benefits of using sporeless kelp sporophytes

The risks of domesticated farmed kelp interbreeding with the wild population include reduction in genetic diversity and adaptation, alteration of population structures and composition, and outcompeting of wild variants by domestic variants (Laikre 2010; Campbell et al. 2019). This potential loss of genetic variance would then reduce the natural gene pool from which kelp breeders can derive desirable traits from future natural variants as well as reduce resistance to disease and adaptability to changing environmental conditions. Therefore, it benefits the environment and the industry to minimise or eliminate the genetic interference of cultivated variants on wild kelp populations. In the marine environment, the consequences of individual spores from a kelp farm escaping into the surroundings are currently unquantified. However, depending on the origins and size of the farmed population and the physical distance from wild populations, there could be a potentially significant impact (Valero et al. 2017; Campbell et al. 2019; Goecke et al. 2020). The likelihood for an escaped cultivated individual to encounter a wild individual and possibly reproduce is higher in nearshore environments than the open ocean, as wild kelp populations are more common nearshore. Currently, the vast majority of global kelp farms are located nearshore. However, if sporeless varieties were cultivated, the chance of any escaped individual establishing itself in the surrounding environment and reproducing would presumably be eliminated. This could enable the responsible cultivation of selectively bred species in any location, subject only to the species' tolerance of the environmental conditions, the carrying capacity of the cultivation site, and regulatory permissibility.

Development of sporeless varieties potentially enables regulatory support of selectively breeding for superior production traits whilst protecting natural populations. If successful, cultivating superior strains could lead to more reliable and productive harvests, which will increase the efficiency and cost-effectiveness of the farming and harvesting processes. Multiple native and non-native species may be grown and inter-cropped to make more diversified farms, increase resilience and productivity, and reduce susceptibility to disease and pests. Farmers would no longer be constrained by the range of temperatures suitable for planting and harvesting within the seasonal production cycles of wild kelp through the selection for traits (e.g., temperature tolerance, resistance to biofouling) that would allow for production for a greater proportion of the year. Altogether, this would support upscaled seaweed farming in becoming a more financially profitable pursuit that also realises socioeconomic, environmental, and climate benefits.

The use of sporeless gametophytes with genetically desired varieties will require farmers to secure new parental material after each harvest as sporeless seaweed cannot be propagated for the next generation. This requirement will propel the development of specialised nurseries/"seed" companies to maintain an array of gametophytes of different genotypes to be planted in different environments. Test farms will be developed for selective breeding and propagating new superior and sporeless strains to continue the robust expansion of a more profitable seaweed industry.

An additional use for sporeless techniques may be the propagation of non-native seaweed species. For instance, *Undaria pinnatifida* (wakame) is among the most promising foods (Yamanaka and Akiyama 1993) as it is full of healthy bioactive compounds and has one of the highest protein contents of kelp species. However, it is also listed among the 50 most invasive species (South et al. 2017). After being introduced accidentally from Japan via oyster shells, French farms were established in the 1980s to farm wakame (Perez et al. 1981; Castric-Fey et al. 1993), but cultivation remains limited due to its invasive potential. Still, wild wakame populations flourished and spread around Europe from Norway to the Mediterranean (Kraan 2017), New Zealand and Australia, North America in California and South America in Argentina (James et al. 2015; Epstein and Smale 2017). We posit that developing sporeless wakame could enable it to be profitably farmed in a range of environments whilst mitigating its invasive potential.

Potential risks of using sporeless kelp sporophytes and risk mitigation

While triploidy renders most gametes imbalanced and incapable of forming a fertile zygote, rare reversions to diploidy are expected by chance. In triploid terrestrial crops, there have been some instances of viable seeds being produced, however, this is very rare (Wang et al. 2016). There have been recorded instances of fertile triploid oysters (Jouaux et al. 2010). There are also remote possibilities that mutations selected for sterility may be spontaneously reversed. By identifying multiple sterility genes and double mutants for that trait, it should be possible to reduce the frequency of genetic reversions to be so low that this technique is stable enough to be used safely in seaweed farms. It is possible that this method may produce more stable sporeless sporophytes than using polyploidy, or that the methods may be complementary. However, further research is required for both methods to understand the frequency of spontaneous genetic reversions.

If there is a possibility of reversion or release of viable farmed meiospores/gametophytes that could reproduce with the wild population, risks can be further reduced by locating the farm in areas of low or no wild kelp populations, such as sandy nearshore areas with no wild populations, the open ocean, or in geographies with winter temperatures suitable for farming but summer temperatures too high for survival in the wild. Meiospore dispersal is naturally limited due to the short time that they are viable (< a day) before they settle, and is largely driven by water currents (Dayton 1985; Norton 1992; Billot et al. 2003; Bartsch et al. 2008). Meiospore dispersal distance varies and is rarely more than 1 km (Fredriksen et al. 1995; Gaylord et al. 2006). However, many of the advantages of upscaled kelp cultivation are maximised when located near coastal areas and locating farms offshore is currently not lucrative due to logistics and high costs. Thus, the most prudent solution is to farm sporeless sporophytes in nearshore locations whilst monitoring the stability of the sporeless trait.

Another risk to be aware of is that of using single clonal cultures as a starting stock. The effects of this can be seen in species such as *Eucheuma* and *Kappaphycus*, the most widely farmed carrageenophytes, where the lack of genetic diversity has resulted in loss of vigour. This has led to a rise in disease and infestations, reduced yields and reduced quality of the carrageenan product (Reddy et al. 2017). In order to avoid a similar scenario, it is important to develop several sporeless kelp varieties to improve hybrid vigour and plant them on rotation or together in the farm to avoid the negative effects of both clonal cultures and monocropping.

Industry regulations

With recent scientific and engineering advancements, the possibility of scaling up seaweed aquaculture is quickly becoming more feasible. However, industry regulations for cultivated seaweed permitting and processing remain in their infancy. For example, most European and U.S. regulations do not yet distinguish cultivated seaweeds from other more common agri- or aqua-cultured species (National Sea Grant Law Center 2023) and biosecurity considerations are poorly integrated (Campbell et al. 2020). Growing public and private interest in the expansion of seaweed aquaculture will likely drive a renewed focus on cultivated seaweed regulations based on sound science with the aim of facilitating a thriving, sustainable seaweed industry compatible with existing ocean uses and conservation. Some steps are already being taken towards this goal, for example, the European Commission proposing action through the Communication ‘Towards a strong and sustainable EU algae sector’, which outlines 23 actions to nurture the industry including identifying optimal seaweed farming sites, advising farmers, and increasing scientific knowledge through studies (European Commission 2022).

As discussed earlier, the development of infertile agri- and aqua-cultured strains has been a critical step in the expansion of many existing food industries. The close monitoring and regulation of factors including the origin, maintained sterility, and exchange of infertile stock seed have been essential to the regulatory permissibility of sterile strains in other industries, and will likely also be critically examined if sporeless seaweed strains are integrated into the seaweed industry (Martinez et al. in prep).

Conclusions

The use of sporeless kelp sporophytes to aid the scale-up of seaweed farms with low environmental risk is a promising and logical next step for the seaweed industry, following in the footsteps of other well-established aquaculture industries dependent on sterility techniques (e.g., grass carp). Research is being undertaken to investigate the viability of different methods to achieve sterility and the outcomes and risks associated with each to ultimately identify suitable biological and regulatory processes that can be applied to seaweed farming. However, more research will be required to reach this goal, accompanied by support and uptake by regulatory frameworks, industry practices, and stakeholder demand. Overcoming these hurdles can pave the way towards an economically and environmentally beneficial seaweed industry across Europe and North America.

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