A concise review of the brown seaweed *Sargassum thunbergii* — a knowledge base to inform large-scale cultivation efforts

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Abstract

Sargassum thunbergii is a brown macroalga endemic to the northwest Pacific. It plays important ecological roles in the structure and maintainance of coastal marine ecosystems. The bioactive compounds extracted from *S. thunbergii* have been extensively documented for potential use in the pharmaceutical and food industries. The species is edible and contains relatively high levels of proteins, minerals and several types of amino acids. The present work compiles recently published literature on *S. thunbergii*, with particular focus on cultivation efforts in China, including the breeding of seedlings and cultivation at sea. A concise review of possible applications is given. Distribution, range shifts associated with past climate change, population genetic structure and connectivity, life history, reproduction and development are all detailed. The review provides important guidelines for future large-scale farming of *S. thunbergii*. This will help aquaculturalists (phyconomists) to meet the expected increases in demand by industrial users. It will also help to conserve natural populations which may be declining due to destructive harvesting and rapid ocean changes.

Keywords Aquaculture; Climate change; Conservation; Cultivation; Ecological adaptation; Genetic diversity

Introduction

Sargassum thunbergii (Mertens ex Roth) Kuntze (Basionym: *Fucus thunbergii* Mertens ex Roth) is a perennial marine brown macroalga, belonging to the family Sargassaceae in the order Fucales. It was first described by the German botanist Otto Kuntze in 1880. This species had some homotypic synonyms such as *Cystoseira thunbergii* (Mertens ex Roth) C. Agardh 1820, *Myagropsis thunbergii* (Meters ex Roth) Kützing 1849 and *Turbinaria thunbergii* (Mertens ex Roth) Yendo 1905 (Guiry and Guiry 2021). *Sargassum thunbergii* also had heterotypic synonyms, e.g. *Fucus swartzii* C. Agardh 1815, *Rhodomela swartzii* (C. Agardh) C. Agardh 1824; *Myagropsis swartzii* (C. Agardh) Kützing 1860 and *Sargassum swartzianum* Yendo 1907 (Guiry and Guiry 2021).

Sargassum thunbergii is one of the most common habitat-forming macroalgae with important ecological roles in coastal marine ecosystems. *S. thunbergii* can accumulate heavy metals and metalloids (e.g., Zinc, Cadmium, Copper and Arsenic) in polluted waters (Wu et al. 2010). It can also assimilate nitrogen and phosphorus in eutrophic coastal waters (Wang et al. 2011).

The bioactive components in *S. thunbergii* have many pharmaceutical and nutraceutical applications. For example, an ethanolic extract of *S. thunbergii* inhibits oedema in mice without acute toxicity, thus presenting a potential remedy for inflammation-related symptoms in humans (Kang et al. 2008). Potential nutraceutical benefits include the reduction of blood sugar and fat, immune system regulation, elimination of superoxide anion radicals (e.g., $O_2^{-}\cdot$), prevention of thrombus formation and of bacterial infections via functional polysaccharides, polyphenols and/or liposoluble compounds (Sun et al. 2018). Alginate, mannitol and iodine can be extracted and these are important raw materials for chemical industry (e.g., in textile production and rubber processing) (He et al. 2011; Sun et al. 2018).

Because of its high nutritional value and low alginate content, *S. thunbergii* is widely used in the food industry. It is rich in protein, vitamins, minerals and umami amino acids. The nutrient composition is comparable with the edible kelp *Saccharina* *japonica* (Areschoug) Lane, Mayes, Druehl et Saunders in East Asia (Table 1) (Tao et al. 2001; Hu et al. 2016). *S. thunbergii* has long culinary history in China, Japan and Korea.

In Chinese marine aquaculture, *S. thunbergii* has been used since the early 2000s as a preferred natural food for sea cucumber and abalone (Han and Li 2005). Juvenile sea cucumber (*Stichopus japonicus*) that had been fed fresh, ground *S. thunbergii* exhibited greater growth rate, survival rate and disease resistance than when fed with other marine macroalgae (Zhou et al. 2010; Guo et al. 2011). The widespread use of *S. thunbergii* has resulted in its over-exploitation in northern China (Liang et al. 2014). This has put considerable pressure on natural resources. Marine farming of *S. thunbergii* in coastal areas will help to both meet the increasing demand of the market and restore declining natural populations and the intertidal habitats they support.

Distribution and range shifts driven by historical climate change

Sargassum thunbergii is one of the most dominant macroalgae endemic to the Northwest Pacific, it is found between middle and lower intertidal levels. Its grographical range currently spans approximately 25° in latitude from Hokkaido, Japan (44°N) to Hainan Island, China (20°N) (Tseng 1983; Titlyanov et al. 2015). *S. thunbergii* finds suitable habitat along the coasts of the East China Sea, Yellow-Bohai Sea, the Korean Peninsula and the Japanese Archipelago (Fig. 1a). The range of *S. thunbergii* in the Northwest Pacific has been influenced considerably by historical climate change (Hu et al. 2011, 2017), particularly the Quasi-100 ky glacial-interglacial cycles triggered by the carbon-climate and other mechanism, in which climate, carbon cycle and ice-sheets interact with each other to produce a feedback that can produce the major observed Quaternary climate variations (Zeng 2006). The periodic, dramatic fall and rise of sea level driven by Paleoclimatic oscillations re-structured coastal topology and connectivity to surrounding habitats, may explain the distribution of other coastal marine sessile species in the Northwest Pacific (Benzie and William 1997). For instance, sea levels in the Northwest Pacific dropped by 120–140 m during the Last Glacial Maximum (20 kya) (Lambeck et al. 2002), leading to the emersion of land mass and the establishment of several marginal sea basins in the Northwest Pacific (Fig. 1b) (Wang 1999; Voris 2000). The South China Sea basin became a semi-enclosed marginal sea and the East China Sea basin was reduced to an elongated Okinawa Trough (Fig. 1b). Species distribution models suggests that lower sea levels during the Last Glacial Maximum considerably shifted the distribution of *S. thunbergii*, with the southernmost boundary of suitable habitats extending approximately to 5°N southward (Fig. 1b). At the same time, the northernmost boundary of *S. thunbergii* retracted to around 35° N (Fig. 1b). When the marginal seas reunited, due to postglacial sea-level rise, *S. thunbergii* populations expanded northwards driven by coastal currents (Li et al. 2017). During the Mid-Holocene (6 kya), *S. thunbergii* populations moved eastwards to the coasts of the Chinese marginal seas and northwards to the coasts of the Korean Peninsula and further to Japan (Fig. 1c). Therefore, climate change since the Last Glacial Maximum and associated coastal environmental oscillations have played a significant role in shaping the present day geographical distribution of *S. thunbergii* in the Northwest Pacific.

Genetic variation and connectivity of populations

Population genetics provides some of the most crucial information to guide sustainable exploitation and conservation of seaweeds with commercial values. Such information includes genetic population structuring and connectivity, the presence of unique gene pools, the genetic relics in pristine habitats and the distribution of genetic diversity within populations. At a regional scale around the Shandong Peninsula in China, four *S. thunbergii* populations showed high genetic differentiation and a clear relationship between genetic and geographical distances (Isolation by Distance model) (Zhao et al. 2007). Subsequently, two genetic lineages along the coast of China were independently identified by two molecular markers (Li et al. 2017a; Liu et al. 2018). There was a clear north to south breakage corresponding to the geographic isolation resulting from the presence of low-salinity water lenses in the expansion area of the Changjiang (Yangtze) diluted water. In Japan, *S. thunbergii* showed a clear genetic differentiation at Single Nucleotide Polymorphism (SNP) loci in double digest restriction site-associated DNA sequencing (ddRAD-seq) between populations from four geographic regions: Kyushu, the Sea of Japan, Hokkaido and Tohoku, and along the Pacific coast from Kyushu to Kanto. The authors also proposed that *S. thunbergii* populations from Kyushu and the Sea of Japan maintained different genetic lineages from those of China and Korea (Kobayashi et al. 2018).

Across the entire Northwest Pacific, *S. thunbergii* populations separated into multiple microsatellite-based clusters (K = 6, Fig. 2a) with a hierarchical genetic structure (Li et al. 2017b), resembling the phylogeographic structure observed for *S. fusiforme* (Hu et al. 2017). This phylogeographic diversity pattern may result from multiple dispersal and vicariance events (Hu et al. 2015; Zhong et al. 2020). *S. thunbergii* populations along the Pacific coasts of Japan (Pops 1–4 in Fig. 2b) show high levels of genetic variation, which could be explained by the maintenance of distinct ancestral genetic variants (Li et al. 2017b). A deep genetic split was further detected between populations in the Yellow-Bohai Sea (Pops 14–27) and East China Sea (Pops 28–35) (Fig. 2b). Based on the species' biogeographic history projected by distribution modelling (Fig. 1b, 1c), we can infer that genetic population variation in the Sea of Japan, and along the Korean and Chinese coasts may be explained by vicariance and ad-mixture of populations that have survived the Last Glacial Maximum along the Okinawa Trough and the South China Sea (Fig. 1b; Hu et al. 2017).

Population ad-mixture can be driven by ocean currents. In the Northwest Pacific, the dominant ocean current system comprises the China Coastal Current, the Kuroshio Current and its branches, the Yellow Sea Warm Current and the Tsushima Warm Current (Fig. 3a). These coastal currents accelerate genetic exchange between *S. thunbergii* populations from different marginal seas, leading to an ad-mixture of different ancestral populations. Molecular analyses clearly showed that gene flow between *S. thunbergii* populations was almost identical to the direction of ocean currents in this region (Li et al. 2017b). In particular, significant gene flow was detected from southern China (Pop 28, Pops 29+30) to the Sea of Japan (Pop 7), via southern Korea (Pops 11+12+13) (Fig. 3b). This suggests that, southern Korea acted as a transition zone across which *S. thunbergii* populations migrated from the East China Sea to the Sea of Japan. The Tsushima Warm Current, originating from the Okinawa Trough, may contribute to genetic connectivity between populations in southern Korea (Pops 11+12+13) and the Sea of Japan (Fig.3). The dispersal of *S. thunbergii* along the Korean coast appears to be mainly driven by the southward Korean Coastal Current and northward Tsushima Warm Current (Fig. 3b). Although microsatellites revealed two genetically diverged clusters in *S. thunbergii* along the coast of China, strong asymmetric gene flow was detected from Pops 25+27 to Pop 28 and from Pop 28 to Pops 29+30, suggesting the China Coastal Current can transport floating marine organisms (e.g. algal fragments) from the Yellow-Bohai Sea to the East China Sea (Fig. 3b).

Life history and reproductive characteristics

Sargassum thunbergii has a haplobiontic life cycle that lacks a gametophyte generation, and the dominant diploid phase is sporophyte (Critchley et al. 1991) (Fig. 4). The gametes are the only haploid phase. The sporophyte of *S. thunbergii* is dioecious, and can reproduce both sexually and vegetatively. Sexual reproduction in *S. thunbergii* is oogamous. Vegetative reproduction is possible through rhizoidal extensions, i.e., new thalli germinate around rhizoid (Fig. 4g).

Sexual reproduction

The age of maturity differs among *Sargassum thunbergii* populations from different sea areas. It increases with latitude and high temperature along the coast of China. Therefore, it seems likely that sea temperature may be a key factor affecting the maturation of *S. thunbergii* (Zhan et al. 2006; Wang and Liu 2007; Zhang et al. 2007). When the male and female sporophytes become mature, the receptacles will grow on the specifically modified laterals (secondary lateral branches) (Fig. 4b). The receptacles are oblong or cylindrical (cigar-shaped), with a blunt tip. They occur singly or in small groups, growing in the leaf (phyllode) axils (Wang et al. 2006).

There are significant differences in shape and size of the receptacles between populations from different geographic areas. Male and female conceptacles are formed on the male and female receptacles, in which the antheridia and oogonia develop, respectively. Generally, the female receptacles are relatively thick and short, about 3–14 mm in length, whereas the male receptacles are more slender, about 10–23 mm in length (Wang et al. 2006; Zhan et al. 2006). However, some female and male receptacles are similar in appearance, and it is not easy to distinguish between sexes when they are not fully mature (Wang et al. 2006). Following fertilization, a diploid zygote is formed by the fusion of an antherozoid with an egg, which germinates immediately and grows into a new sporophyte (Fig. 4c, 4d, 4e). At the same time, segmentation of the zygote/germling proceeds rapidly (Critchley et al. 1991).

At maturity, the female receptacles are slightly rough in texture due to the newly released eggs or fertilized eggs (zygotes) attached to the surface (Fig. 5a). This phenomenon resembles the “incubation” of germlings in *Sargassum muticum* described by Nicholson et al. (1974). The surface of male receptacles remains smooth (Zhan et al. 2006). The male and female conceptacles are located within the male and female receptacles, respectively. Self-fertilization does not occur in *S. thunbergii*. The male conceptacles are relatively sparse, with about 80–120 conceptacles on each receptacle. The diameter of the conceptacle ostiole is about 84–150 μm (Wang et al. 2006; Pan et al. 2007; Wang et al. 2007). The female conceptacles are densely clustered compared to conceptacles on the male, but the total number is smaller than that of male, with about 60–90 conceptacles on each female receptacle (Wang et al. 2006; Wang et al. 2007). The ostiole of the female conceptacle has a larger diameter of about 130–200 μm. The ostiole diameter in the upper part of the receptacle is slightly smaller than that at the receptacle base, but the conceptacle density in the upper part of the receptacle is greater than that at the receptacle base. The maturity of conceptacles decreases from the base to the top (acropetal maturation) (Wang et al. 2006; Wang et al. 2007).

The conceptacle develops from a single superficial cell in the receptacle which is called the conceptacle initial. This cell is flask shaped, larger and has a more prominent nucleus than the adjacent cells. The initial cell divides more slowly than the surrounding cells, and thus becomes invaginated. The initial cell divides transversely into two cells, known as the lower basal cell and the upper tongue cell. The tongue cell divides transversely to create a small filament which later disintegrates. The basal cell makes the fertile layer of conceptacles by continuous vertical division (Sun et al. 2007).

The cells in the fertile layer of the female conceptacle develop into oogonia (Sun et al. 2007). The oogonial initial cell on the fertile layer of the female conceptacle divides transversely, resulting into a small, lower stalk cell and a large, upper oogonial cell. The oogonial cell enlarges and forms a spherical oogonium. During oogenesis in *S. thunbergii*, meiotic and subsequent mitotic divisions occur without cytokinesis. Therefore, all eight nuclei remain throughout the maturation process of egg. After plasmogamy, one of the eight fuses with a sperm nucleus and the other seven then degenerate gradually during the development of the zygote (Nagasato et al. 2001; Zhao et al. 2008). This cell forms a single ovum, which will fuse with the nucleus of an antherozoid when fertilization occurs (Pan et al. 2007). The mature oogonia protrude from the conceptacle through the ostiole, but they remain attached to the base of the conceptacle by a long gelatinous stalk. When the oogonia are entirely released from all the conceptacles of a receptacle, the oogonia can completely wrap the surface of the receptacle (Wang et al. 2006; Pan et al. 2007; Wang et al. 2007). This incubation period may confer an advantage for *S. thunbergii* to succeed in germling production (Nicholson et al. 1974). Unfertilized eggs showed strong adhesion ability in an indoor nursery and can detach from the receptacle within 3 days; fertilized eggs showed weak adhesion and can detach within 1–2 days (Zhan et al. 2006; Wang et al. 2007). Increasing water temperature can stimulate egg release (Zhan et al. 2006). Receptacles of sufficient maturity can be ovulated on the same day when placed in a sea water that exceeds the temperature of natural sea water by 3–4°C. In the field, this would take 3–4 days (Zhan et al. 2006; Zhang et al. 2007).

The cells on the fertile layer of the male conceptacle can divide transversely to form the lower stalk cells and the upper antheridial cell (Sun et al. 2007). The stalk cells undergo multiple transverse divisions to form more stalk cells and antheridial cells. The antheridial cells enlarge and round to form spherical antheridia. The diploid nucleus of the antheridial initial undergoes meiosis, followed by repeated mitotic divisions, forming 32–64 haploid nuclei. The nuclei then accumulate cytoplasm and form haploid antherozoids. The antherozoids, pear-shaped with two laterally inserted flagella, are released into the water column after the gelatinization of the outer wall (Sun et al. 2007).

Fertilization and zygote development

In indoor culture of *S. thunbergii*, the ovulation time of the female receptacles is earlier and shorter compared with the male receptacles (Wang et al. 2007). Females can ovulate in the absence of males, whereas the males will often not discharge any antherozoids without females, implying that the antherozoid discharge may be induced by chemical substances released during the ovulation process (Wang et al. 2007). After being discharged from the female conceptacle, the eggs adhere to the outer surface of the receptacle until fertilization (Fig. 5a). After being released, the antherozoids swim to the egg with the help of the flagella and unite with it to form a diploid zygote.

About 2–4 hours after fertilization (Liu et al. 2006), the zygote undergoes the first horizontal division to form upper and basal cells, and the basal cell divides again to form a smaller cell at the base, which will further differentiate into rhizoids (Wang et al. 2006; Pan et al 2007; Zhao et al. 2008). The upper cell divides once, approximately every 2–4 hours, and after multiple vertical and horizontal divisions, a pear-shaped embryo sporophyte is formed (Wang et al. 2006; Pan et al 2007; Zhao et al. 2008) (Fig. 5b-5e). When the embryonic sporophyte grows and develops about 16 rows of cells, that is, about 20–48 hours after fertilization, the basal cells form 4–8 protrusions (Fig. 5f), which are the initials of the rhizoids (Wang et al. 2006; Pan et al 2007; Zhao et al. 2008). The protuberances grow continuously, and the rhizoid quickly exceeds the length of the sporophyte thallus (Pan et al 2007).

The emergence of the rhizoid indicates the formation of an intact young sporophyte (Fig. 5g), with the ability to attach when falling off from the receptacle. The embryonic sporophyte develops from the fertilized egg within 24–72 hours on the surface of receptacle, after which it detaches from the receptacles generally from around midnight to the early morning of the next day. Mechanical disturbances, such as agitation, are beneficial to detachment (Sun et al. 2010; Zhang et al. 2007). However, some fertilized eggs or embryonic sporophytes, and even the newly produced rhizoid can remain attached to the surface of the receptacle, and finally detach together with the receptacle from the thallus (Wang et al. 2007). The shed embryonic sporophytes, when attached to a suitable substrate with the rhizoid, can further grows into a large sporophyte. The whole process can be seen in the form of an animation here: https://www.youtube.com/watch?v=Teeyar2B310&feature=youtu.be

Seedling in the nursery

In recent years, wild resources of *S. thunbergii* have been drastically depleted due to global climate change and habitat destruction (personal observations), and are, thus, becoming less available for the species' important economic and ecological applications. However, the artificial cultivation of *S. thunbergii* requires large quantities of seedlings. These were mainly obtained by manual collection from natural populations. As a consequence, the wild resources of *S. thunbergii* have been devastatingly damaged in China. In order to protect the wild resources an artificial seedling production system has been developed (Fig. 6) (Sun et al. 2007; Li et al. 2009; Zhang et al. 2012).

Facilities and equipment for artificial seedling rearing

Seedlings of *S. thunbergii* are generally bred in rectangular concrete ponds at ambient temperatures (18–23℃) and under natural light (avoiding direct sunlight) (Sun et al. 2007; Li et al. 2009). Nurseries for cultured macroalgae, e.g., *Saccharina* spp. and *Pyropia* spp., can be used (Fig. 6a) in addition to special nurseries dedicated to *S. thunbergii*. Glass greenhouse enclosures need good ventilation. Glass or transparent

Fibre Reinforced Plastic (FRP) should be installed on the roof to ensure good lighting in the nursery. In the nursery, ponds can be of variable size but the depth should not exceed about 0.5 m for optimal operation. In addition, a seawater treatment system is required, including and sedimentation tanks and sand filter facilities.

The attachment substratum for the juvenile seedlings of *S. thunbergii* – called the seedling collector (Fig. 6b) can be of stones, shells, bamboo, wooden boards, cement boards, palm-fiber rope and various synthetic fiber materials (e.g. polyethylene or vinylon) (Liu et al. 2017). On account of the efficiency, ease of operation and cost, the most commonly used seedling collector is made by weaving vinylon cloth strips (approximately 2 cm in width) onto a plastic frame with a length of 1 m and a width of 0.5 m. This type of seedling collector provides uniform density and firm attachment for seedlings, and is convenient to use in practice (Sun et al. 2007; Li et al. 2009).

Selection and treatment of parental thalli

Wild or artificially cultivated populations can be used as parental thalli for artificial seedling rearing (Fig. 6c) (Sun et al. 2007; Li et al. 2009; Zhang et al. 2009). The individuals providing best parental thalli are strong and healthy, presenting an abundance of lateral branches and mature receptacles. The level of maturity directly determines the success of seedling breeding (Sun et al. 2007; Li et al. 2009). Upon being wrapped by the protruding eggs, the surface of the female receptacle begins to secrete a large amount of mucus, indicating that the receptacle is ready for seedling collection. Temperature has been proven to be a key factor affecting the maturation of *S. thunbergii*. Thus, seasons of growth and production of *S. thunbergii* vary significantly among regions due to temperature shifts in the Northwest Pacific (Koh et al. 1993; Yatsuya 2008). Therefore, it is necessary to select different locations to collect sufficiently mature individuals at the appropriate time of the year, for harvesting germlings for artificial seedling rearing.

Collection of fertilized eggs

Each square meter of the seedling collector generally needs 0.5–1 kg of parental thalli to provide sufficient propagules, and the weight ratio of female to male parental thalli should be about 6–10:1. Before the collection of fertilized eggs, the parental thalli are rinsed 3–4 times with filtered seawater. Thalli can be dried in the shade for several hours to promote the discharge of eggs. If there are already attached eggs on the receptacle surface, it is not necessary to dry in the shade (Wang et al. 2006; Zhan et al. 2007; Li et al. 2009). There are two methods for fertilized egg collection as outlined below (Li et al. 2009).

Direct collection of fertilized eggs: The seedling collectors are laid in the nursery ponds sterilized by chlorinated lime, and filled with fresh sand-filtrated seawater to a depth of about 30 cm. The parental thalli are laid evenly on the seedling collector, so that the fertilized egg or embryonic sporophyte will fall off naturally and attach to the seedling collector. During the collection, frequent turning the parental thalli can speed up the shedding of the fertilized eggs and facilitates the uniformity of sporophyte attachment. This process will take about 1–2 days, then the parental thalli are taken out the pond. The fertilized eggs or embryonic sporophytes are not firmly attached at this moment. Thus during water replacement, water flow must remain slow in order to prevent the sporophytes from being washed away.

Spraying of fertilized eggs: The parental thalli are concentrated in a nursery pond until the fertilized eggs are shed. The shed eggs and embryonic sporophytes are collected with a 300-mesh sieve, and sprayed onto the seedling collectors according to the planned seedling density (Fig. 6d). Good seedling results have been demonstrated when the density of fertilized eggs is controlled between 8 and 20 on each seedling collector. This can be discerned under a microscope with100× magnification.

Indoor rearing of seedlings

Temperature, light and nutrients are important environmental factors affecting the growth of *S. thunbergii* seedlings. Studies have shown that *S. thunbergii* seedlings grow fastest at 18–23℃ (Liang et al. 2012; Ma et al. 2013; Wu et al. 2015). The juvenile sporophytes grow well at a range of light 40–160 μmol photons/(m^2·s) (Ma et al. 2013). When the seedlings reach a length of 3–5 mm, the demand for nitrogen increases. At a nitrogen concentration of 2–10 mg/L, the growth rate maintains an upward trend. The optimal phosphorus concentration for the growth of seedlings is 0.4 mg/L, with the optimal ratio of nitrogen-to-phosphorus at 20:1 (Ding et al. 2014). *S. thunbergii* seedlings can survive at a salinity of 21–40 PSU, but grow best at 27–30 PSU (Zhan et al. 2006).

In the nursery, *S. thunbergii* seedlings are best cultivated with seawater that has been allowed to settle for 24 hours and filtered through sand. The water temperature should be 18–23℃, and the sunlight intensity is should be kept below 300 μmol photons/(m^2·s) by manipulating windows and/or roof shades. Two or three days after the collection of fertilized eggs, seedling collectors should be washed daily under a gentle water current. After 7 days, the seedling collectors are washed using a pressure water jet with the pressure from weak to strong, regulated to avoid the detachment of the seedlings from the collectors. The washing operation should be carried out once every other day (Zhan et al. 2006; Li et al. 2009). The washing of seedling collectors is one of the most important tasks for the indoor culture of *S. thunbergii* seedlings. This operation can not only remove other competitive organisms and sludge on the seeding collectors, but also improves the attachment of *S. thunbergii* seedlings (Zhan et al. 2006; Li et al. 2009; Zhang et al. 2012).

Seedling transplant from nursery to the sea

After a period of indoor rearing, the indoor conditions can no longer fully meet the growth requirements of *S. thunbergii* seedlings, and it is necessary to transplant the seedlings into the sea (Li et al. 2009). Production practice has shown that if the seedlings are transplanted into the sea too early, the rhizoids of the seedlings remains too weak to support attachment, resulting in the loss of seedlings at sea. However, if the seedlings are transplanted too late into the sea, their growth will be retarded (Li et al. 2009). Rearing practices came to the consensus that the optimal duration for indoor rearing of *S. thunbergii* seedlings is 10–20 days (Zhang et al. 2007; Li et al. 2009). When the seedlings have developed more than 15 rhizoids, and the average thallus height is 2 mm or more, they are ready to be deployed at sea. The seedling collectors are hung horizontally on a floating raft at a water depth of 30 cm (Fig. 7a, Figs. 8a, 8b). After the seedlings have been newly transplanted from the nursery, the seedling collectors provide empty space for the attachment of various competitive seaweeds, sludge, and invertebrate larvae. If they are not cleaned up in time, the seedlings will be covered, hindered in growth, and can even detach and die (Li et al. 2009; Zhang et al. 2012). Therefore, it is essential to wash the collectors from time to time. This is best done using high-pressure sea water jets.

Artificial cultivation at sea

Sea areas suitable for the cultivation of *S. thunbergii* should not be influenced by urban sewage, industrial waste or fresh water from rivers. The water depth at high tide must exceed 3 m, the current velocity must exceed 0.6 m/s, and the salinity must go up 20 PSU (unpublished data). Floating rafts are the main facilities for *S. thunbergii* cultivation (Fig. 7b; Figs. 8c, 8d), mainly comprising anchors, anchor ropes, floating ropes, cultivating ropes, and floats (floating balls, floating bamboos, etc.).

When the cultivated seedlings of *S. thunbergii* exceeds 2 cm in length, they can be removed from the seedling collector and clamped to the cultivating ropes (Fig. 8d). The distance between seedlings shall be about 8–10 cm. The cultivating ropes are hung off a floating rack for cultivation (Fig. 8d), with a space of about 1 m between two ropes. The seedlings then begin to grow rapidly, and form disc-shape rhizoids that can firmly attach to the cultivating ropes. At the end of autumn, several branches sprout from the rhizoid, forming a cluster of thalli. After the fast growing season in autumn, the growth rate of *S. thunbergii* slows down due to the low temperature in winter (Chen et al. 2016). In northern China (e.g. Shandong Province), the cultivating ropes need to be lowered to 50–100 cm in order to improve the winter survival rate of *S. thunbergii*. In spring, as the temperature rises, the growth rate of *S. thunbergii* increases again. From May to June, *S. thunbergii* gradually matures, and is ready to be harvested when the thallus reaches a length of about 2 m (Chen et al. 2016). The daily management of *S. thunbergii* cultivation mainly includes: i) inspection of the cultivating raft structure to ensure its efficiency and safety; ii) inspection of the seedlings to determine if they have detached and/or lost due to waves or other reasons. If this happen, they should be replaced by new seedlings; iii) adjustment of the water depth determined by the floats and anchor ropes according to the transparency of the sea water.

Prospects for the cultivation of *S. thunbergii*

Cultivation on shallow sea rafts can increase the growth rate and, thus length and fresh weight of *S. thunbergii* by about three times compared with wild individuals growing on natural rocks (Zhou et al. 2005). Therefore, the increasing commercial demand for this species can be best satisfied by artificial cultivation. The recovery of declining natural populations has the highest potential when parental thalli originate from cultivated instead of wild grown thalli, and when seedlings are cultivated for 2–3 consecutive years (Yuan et al. 2006). If the rhizoids are left behind after harvesting, *S. thunbergii* can regrow from them. This ability emphasises the exceptional potential that exists for the artificial cultivation of *S. thunbergii*.

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Figure captions

Fig. 1 Ensemble Species distribution maps for *Sargassum thunbergii* for the present (a), the Last Glacial Maximum (b) and the Mid-Holocene (c).

Fig. 2 Genetic structure of *Sargassum thunbergii* populations based on microsatellites. (a) Each vertical column showed the probabilities (partitioned into sections along the y-axis) of an individual to be assigned to six different genetic clusters that are represented by different colors. (b) Geographic representation of the associated probability of assignments (K = 6) for each locality in pie charts. ID numbers for the populations are indicated in the circles.

Fig. 3 The major oceanic current systems in the Northwest Pacific (a) and gene flow estimated between adjacent *Sargassum thunbergii* populations based on nine microsatellites (b).

Fig. 4 The life history of *Sargassum thunbergii*. a: sporophyte; b: mature male and female receptacle; c: eggs on the female receptacles; d: cell division of the zygote; e: embryo sporophyte with rhizoid; f: seedling attached to the substrate; h: young sporophyte; g: new main axis grown from the rhizoid.

Fig. 5 The sexual reproduction of *Sargassum thunbergii*. a: female receptacle with eggs or zygotes; b: fertilized egg; c-e: cell division of the zygote; f-g: embryonic sporophyte with rhizoids.

Fig. 6 Artificial rearing of *Sargassum thunbergii* seedlings in nursery. a: ponds in the nursery; b: seedling collectors; c: parental thalli laid on the seedling collectors; d: samples of young seedlings at different densities attached to the seedling collector.

Fig. 7 Schematic diagram of a floating raft for rearing seedlings (a) and for growing cultivars at sea (b). (a): 1, anchor rope; 2, floating bamboo; 3, anchor; 4, floating rope; 5, seedling collector. (b): 1, floating rope; 2, anchor; 3, anchor rope; 4, floater; 5, cultivating rope; 6, thalli of *S. thunbergii* hung on the cultivating rope.

Fig. 8 Photographs of the seedlings (a, b) and cultivation of advanced germlings (c, d).

Table 1 Proximate composition of *Sargassum thunbergii* in northern China, including the comparison with the most well-known edible kelp *Saccharina japonica* in East Asia (after Hu et al. 2016; Cao et al. 2017)

Composition	Content (mg/g dry weight)		Composition	Content (mg/g dry weight)	
	S. thunbergii	*S. japonica*		*S. thunbergii*	*S. japonica*
Protein	142-194	87-162	Asp*	11.3-14.8	19.8
Crude fat	1.700-60	2.000-15.400	Ser*	4.9-6.0	2.78
Carbohydrate	590-656		Glu*	31.1-43.9	28.7
Crude fiber	44	98	Gly*	5.0-7.5	3.14
Vitamin C	0.206	0.110	Ala*	6.5-13.3	4.81
Ca	2.600	2.900	ΣUAA	71.2-74.8	59.19
K	30.600	42.700	EAA	32.7-58.8	15.98
P	1.100	1.900	HEAA	6.4-10.2	4.22
Sr	0.885	0.340	TAA	135.4-145.8	80.01
Zn	0.028	0.013	ΣSFAs (%)	29.91-36.00	43.76
Cu	0.014	0.007	ΣMUFAs (%)	18.33-24.78	25.00
Mn	0.077	0.012	ΣPUFAs (%)	29.42-41.77	20.69

*UAA, umami amino acids; EAA, essential amino acids; HEAA, half-essential amino acids; TAA, total amino acids; SFAs, saturated free fatty acids; monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids