Results of Cultivation of Japanese Kelp (*Saccharina japonica*) in Primorsky Krai, Russia

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Abstract: Animals and plants, living near human settlements in the three climatic zones, accumulate substances that allow them to resist extreme environmental factors. By consuming these plants and animals, people strengthen the immune system that also facilitates their existence in harsh conditions. Many of the world known species appreciated for their medicinal properties inhabit Primorsky Krai, which is located in three climatic zones. On land, these are plants of the family of Araliaceae, including the well-known ginseng; in the sea, the Japanese sea cucumber and brown algae, including the Japanese kelp *Saccharina* (= *Laminaria*) *japonica*. This publication provides the results of cultivation of commercially valuable Japanese kelp by several technologies at sea-based farms in Russia.

Keywords: Three Climatic Zones, Health Food, Forced Cultivation, Japanese Kelp, *Saccharina* (= *Laminaria*) *japonica*

1. Introduction

Primorsky Krai of Russia has unique climatic conditions. On its territory, there are three climatic zones: boreal, subtropical, and maritime. The boreal climatic zone has arisen due to the proximity of Siberia with its lowest temperatures in the northern hemisphere. Besides low temperatures, this region is distinguished by such adverse factors as strong winds and the high humidity of the air even during winter season. Therefore, those animals and plants which can survive this climate acquire high nutritional and medicinal value. Chinese pharmacists readily use them for production of medicines. Among plants, roots of ginseng, eleuthero, magnolia-vine, golden root (*Rhodiola rosea*), and dandelion are used most frequently for manufacturing medicines. Even a tincture made from roots of a plant of the aralia family, cultured in my personal garden, exerts a favorable effect on my health. Among marine organisms, Japanese kelp *Saccharina japonica* (J.E. Areschoug) and Japanese sea cucumber are widely used in medicine.

In the countries of the Asia-Pacific region, algae have long been one of the most important seafood items. According to Wamyosho, the most ancient Chinese-Japanese dictionary,

which was edited by Emperor Daigo (897-930) in Japan at the early stages of the Heian (794–1185 A.D.) period, a total of twenty one species of marine algae, including green, brown and red ones, were used as food those times [1, 2]. At present time, algae are used not only as a food but also as drugs because they contain polysaccharides [3], which constitute 40–80% of dry weight in brown algae, depending on species [4] and consist of water-soluble laminarans (1,3- β -D-glucan), fucoidans (highly sulfated α -L-furan), and alginic acids [5], with the latter being the most important component [6]. Drugs derived from kelp polysaccharides increase the concentration of INF- γ and L-1 β in blood that stimulates the differentiation of immunocompetent cells in people with a high level of T-helper cells, cytotoxic lymphocytes, and natural killers blood [7]. Algae contain also ascorbic acid [8], unsaturated fatty acids [9, 10], and organic iodine [11, 12, 13]. In addition, marine algae are a source of biologically active substances such as chlorophyll derivatives, sulfated galactans, fucoidan, glucans, dietary fiber, phenolic compounds, enzymes, plant sterols, vitamins, carotenoids, macro- and trace elements [14]. Due to this complex of useful elements, brown algae can be used for prevention and treatment of endocrine diseases [11, 12, 13], cancer [15, 16], caries [17], as well as for excretion of heavy metals and radionuclides [18]. The anticoagulation, contraceptive, and

other activities typical for fucoidans are also studied [19].

One of the new directions is the use of macrophytes as plant biostimulants [20]. Due to the constant problem of fuel shortage, algae have long become a source for the biofuel production [21, 22, 23, 24, 25, 26]. The expansion of cultivation of valuable fish species in net-pens has created the necessity of their cultivation in polyculture with algae [27, 28, 29]. Currently, not only traditional consumers of “wild” algae (such as the USA, Australia, and the EU) [28, 30], but also the Latin America countries [31] have shown an interest in cultivation of macrophytic algae. They became cultivated became not only for food, medicinal, and industrial purposes, but also for restoration of previously disturbed natural communities. They are secretor of organic and inorganic materials, and as a substrate for attachment [32], food [33, 34], and shelter for many species of algae, invertebrates, and fish [35]. The process of degradation of a kelp thallus results in accumulation of substances that increase the activity of digestive enzymes in sea urchin *Strongylocentrotus intermedius* that makes the algae more digestible and raises its nutritional value [36]. Many species of algae can be used for feeding livestock [37]. By absorbing various trace elements, macroalgae can be an indication of their presence in seawater [38], and by absorbing the greenhouse gas, CO₂, they contribute to slowing down the global warming and reducing the acidification of seawater [25, 39]. Due to the ability of Japanese kelp to accumulate heavy metals in its thallus [40], sea-based kelp plantations can be used for cleaning the waters adjacent to settlements. A twenty-hectare farm of the *Saccharina japonica* can support a city with a population of 11,000 [41].

In current conditions, stable obtaining of product of the marketable size is impossible without artificial reproduction. Currently, algal aquaculture includes a significant proportion of organisms cultivated in the world (~19 million metric tons) to the total price of ~\$5.65 billion USD [42]. In Asia, China produced the largest number of aquatic plant species, approximately 12.8 million tons, in 2012; the second- and third-largest producers are Indonesia and Philippines, where about 6.5 and 1.8 million tons were obtained, respectively [43]. The most promising method of cultivation of *S. japonica* with the use of summer sporeling method, developed in China in 1955 [44], and the forced method, developed in Japan in the 1966s–1970s [45, 46, 47, 48] allow farmers to obtain marketable products within one year. After mastering the advanced technology, the studies on improving the cultivation processes [49, 50, 51] and finding the methods of disease control [52, 53] began abroad. The positive results, obtained in the neighboring countries with the help of this method, stimulated its testing in Russia also [54, 55]. By the moment of launch of the farm for cultivation of early sporophytes of Japanese kelp at the village of Glazkovka (Primorsky Krai) (station 8, Figure 1), the only technology of cultivation known in Russia was the Japanese one. According to this technology, all water for filling the 100-liter tanks was centrifuged and then sterilized, and thus, for successful growing of seedlings the

water temperature of 15°C was optimal [47]. However, in Glazkovka, large pools with the volume of 4.7 m³, connected by three into a single module, were used for growing seedlings, as after the algae were to be used for growing salmon fry. These volumes of water could not be centrifuged and sterilized, and the low-power refrigeration unit was not able to cool the water to a temperature below 8°C. It was increased to 12°C and above by the air and the hot illumination lamps DRL-400 that stimulated bacterial growth in the water and caused diseases of sporophytes. For two years (1982–1983), the mortality of sporophytes in the pools of Glazkovka reached 50%, and about half of the remaining ones died in the sea during the adaptation period. In 1984, about 75% were lost in the pools, and the rest died during the adaptation. By the summer of 1985, the nylon ropes with inserted in them fragments of strands with seedlings, which were distributed over 6 ha of the farm, all appeared to be without sporophytes. At the same time, the part of the seedlings transported to the Stark Strait, off Popov Island (42°58'20"N; 131°52'49"E (station 7, Figure 1), and planted onto 6 mm rope there, reached the marketable size in the summer of 1985. By that time, Chinese researchers had published a work that reported about the disease of kelp seedlings caused by Gram-negative bacteria of the genus *Pseudomonas*. To reduce their harmful effects, sporophytes should be grown in the water having a temperature of 8–10°C and with erythromycin dissolved to a concentration of 0.5 units/mL [53]. The use of this technique allowed us to increase the survival of young sporophytes in the pools [56], and the surplus of seedlings were given to other algal farms. As a result, the total yield of the three algal farms reached 5,000 t wet weigh and exceeded the modern natural stock of Japanese kelp in Primorsky Krai [57].

The basic principles of the Chinese technology of cultivation of young kelp are as follows: sporophytes are obtained in pools, then, after the sea water temperature declines to 17–20°C, they are either transferred to a open-water farm to reach 10–15 cm in length [58], or grown in shore-based greenhouses to 15–20 cm, then are woven into ropes in bundles by 30 sporophytes per 2 m of rope, and relocated to the farms in November [59]. There, they reach the commercial size within less than a year. However, our algal farms are located at more northerly latitudes than those in China, the air temperature here falls below 0°C in November, and sporophytes cannot reach 15–20 cm even in the sea. Therefore, we have to grow sporophytes by the Japanese technology: by weaving pieces of strands with seedlings (fragments) into a nylon rope. To optimize the technology of growing, we have been experimenting with different distances between the fragments, as well as looking for the most optimal environment for seedlings.

The goals of the present study were to find the environment optimal for artificial reproduction of young sporophytes and to develop the most rational technology of their rearing in the sea.

Figure 1. Study area, sites of collection of mature sporophytes, and locations of sea-based plantations: (1) Klikova Bay, (2) Minonosok Bay, (3) Cape Nizhny, (4) Reid Pallada Bay, (5) Cape Gamov, (6) Verkhovsky Islands, (7) Stark Strait, and (8) Kit Bay.

2. Material and Methods

This paper provides the results of cultivation of commercially valuable Japanese kelp by several technologies at sea-based farms in Russia. The goals of the present study were to find the environment optimal for artificial production of young sporophytes and to develop the most efficient technology of their breeding in the sea. To achieve these goals, experiments on the forced cultivation of *Saccharina japonica* in pools with controlled environmental parameters were set up in the village of Glazkovka and in the nearest Kit Bay (station 8, Figure 1) (43°03' N and 134°12' E) in 1985–1987, as well as in the village of Possiet and the nearest Reid Pallada Bay (station 4, Figure 1) (42°32' N and 130° 55' E) in 1988–1990 and in 2005–2006. In Possiet Bay, Reid Pallada Bay, we conducted in 1987–1990 experiments on cultivation of two Japanese kelp races from maternal thalli collected from the waters off the Gamov Peninsula (Possiet Bay) (42°36'10"N; 131°10'55"E) (station 5, Figure 1) and the

Verkhovsky Islands (Ussuri Bay) (42°56'6"N; 131°48'12"E) (station 6, Figure 1). In Glazkovka, the pools with a closed water circulation had a volume of 4.7 m³ and were combined by three into a single module. They were filled with continuously running cooled of sea water and air for aeration leaked on the tubes laid on a bottom. The rate of water change in the pools was constantly maintained at the maximum level (16–20 volumes per day). Aeration was also the highest: the air flow for the three pools was 840 L/min. The water in the tanks was illuminated with white-light lamps for 12 hours a day.

For spore seeding and growing of seedlings, stainless-steel frames 60 × 40 cm were used. A nylon string with a diameter of 1 mm and a length of 400 m was wound around a frame. All frames were soaked in seawater for 5 days and then in fresh water for the same period.

In 2005, in the village of Possiet, we set up an experiment on growing the Japanese kelp sporophytes in ten 100-liter tanks, floating in the pool with cooled sea water. Water in each of them was aerated. One spore-seeded frame was

placed into each tank. These frames are treated in different ways: six frames were boiled in fresh water (variant no. 2), and, after drying, four of them were fired with a blowtorch (variant no. 1), as described in [50]. The two unboiled frames (variant no. 3) were also fired, and the remaining two frames were unboiled and unfired (variant no. 4). In two tanks, the water was treated with antibiotics (nos. 1 and 2); the water in

the tanks 2 and 10 was pasteurized by heating to 90°C for 30 min. In three tanks (nos. 3, 8 and 9), 10 individuals of two-year-old of the Pacific mussel *Mytilus trossulus* was placed. To verify the positive effects of mussel metabolites on growth of sporophytes, no nutritional supplements were introduced into the tank no. 8. The scheme of the experiment is shown in Table 1.

Table 1. The scheme of experiment with frames placed in 100 l tanks.

№ tanks	antibiotic	pasteurization	frames variant	mytilus, 10 ind.	nutritional supplements
1	+	-	1	-	+
2	+	+	1	-	+
3	-	-	1	+	+
4	-	-	3	-	+
5	-	-	4	-	+
6	-	-	3	-	+
7	-	-	4	-	+
8	-	-	2	+	-
9	-	-	2	+	+
10	-	+	1	-	+

The spore suspension was obtained from maternal thalli, taken from a two-year cultivated of Japanese kelp. The time of zoospore release stimulation at a temperature of 15–16°C and a humidity of 80–85% was 9 hours (usually from 12 to 21 hours). After the stimulation, the thallus was placed into a fiberglass tray of 0.9 m³ and filled with seawater. The thalli were kept in the tray until an examination of the suspension under a microscope at the magnification of 120× showed at least 3–5 actively moving zoospores in the view field. As a rule, keeping the thalli in the tray lasted for 2 hours. The obtained suspension was filtered into another tray with frames through a double layer of mill sieve with the mesh of 120 µm (Figure 2). The zoospore suspension reached 1/3 to 1/5 the volume added to the frames. The maximum number of the spore-seeded frames in the tray reached 84. The water

temperature during spore seeding was 18–21°C. The number of spores in a drop of suspension from the tray with frames was 23 at the magnification of 84×. After 18 hours, the density of settled spores reached 200 at the magnification of 100×. After that the frames were transferred to the pools with running sea water and distributed by 28 frames per one pool (each frame was at a distance of from 15 cm from another). The surge of the water temperature during this operation reached 6.2°C. After transferring the frames, the water temperature in the pools was decreased to 11.5°C at a rate of 0.5°C per day; the illuminance was increased from 2000 to 8000 lx by lowering the lamps to the water surface every 5–7 days. Depth of immersing of frames amount up 5 sm. In 1986 to prevent drying of the frames, they were suspended to the floating frame.

Figure 2. Settlement of zoospores on frames with strings. Pools in the village of Glazkovka.

During the 30-day period of keeping the frames in the pools, seedlings were fed with nutritional solution E-S, described in [60] and modified by [46]. In 1986, to increase the rate of sporophytes' growth, vitamins were used according to [61]. Due to the shortage of thiamin, its concentration was 5.25 times as low as the recommended one. Introduction of nutritional supplements was done four times (every 7–8 days) per one culturing cycle. The concentration of the first nutritional solution was 1: 100; all the following ones, 1:50. In 1985, the solution was not clarified; in 1986, either hydrochloric or acetic acid were added for clarification by lowering the pH to 7.8.

In 1985, an attempt to treat sporophytes directly in the pools was made 22 days after the spore seeding. For this, erythromycin was introduced into the pools at a concentration of 0.5 units/mL, and the temperature was lowered to 10.5°C. The reduction of diatom algae in the pools was achieved by vigorous shaking the frames every 2–3 days, and to create a uniform illumination, the frames were turned by 180° once a day.

In the process of growing the sporophytes, the water temperature and illuminance were measured daily in three places of each pool. In addition, water samples to determine the salinity, level of total and mineral phosphorus, ammonia and nitrite nitrogen, and oxygen were taken from four points of the module. Two or three times a month the same measurements were carried out in the sea. The length and width of sporophytes on spore-seeded glasses and strings was measured two or three times a week.

Within a month after the spore seeding, the frames were transferred to the sea in parts. The maximum surge of the temperature when transferring the frames was 6.8°C. They were immersed to a depth of 5–9 m. After the period of seedlings' adaptation in the sea, which lasted for at least 10 days, the frames were transported back in small parties. The strings were cut into 2–3 cm long pieces and woven into a nylon rope of 6 mm in diameter and 6 m in length. The distance between the fragments was 15, 20, and 30 cm. The ropes were suspended vertically on a horizontal ropes with the intervals of 50 cm. A part of the largest thalli were woven into the rope in bundles by 3–4 ones every 15 cm. Due to the high complexity of this process, only 20 ropes with thalli were made. After that, the horizontal ropes were submerged to a depth of 4 m. In March of the following year, the thalli from the fragments woven into the rope with 15-cm intervals were transplanted onto new ropes with bundles of 3–4 algae every 15 cm. The area of the experimental farm was 1.5 ha.

In 1986, six ropes were taken from the farm every month, from February to November, to count the total number of thalli and measure length, width, thickness and weight in 50 of them. In late October, the concentrations of dry matter and minerals were measured in thalli. The statistical processing of the materials was carried out in STATISTICA 6 (StatSoft Inc., Tulsa, Oklahoma, USA). The values of regression analysis were tested at $\alpha=0.05$.

3. Results and Discussion

3.1. The Results of Cultivation of Japanese Kelp in Pools

When the water temperature in pools was 12–15°C, zygotes of Japanese kelp appeared on the glass on the 12th day after spore seeding during two years. Similar growth rates as in Europe at *Laminaria saccharina* were recorded by [62]. The strings in the pools were in different conditions. On the strings placed closer to the water canal, sporophytes were large; the largest ones were found on the strings at a distance of 60 cm from the site of water fall (Figure 3). At a distance of 165 cm from the water fall, there was a strong air supply, and sporophytes on the strings closer to the aerator formed the third peak of growth rate (Figure 3). For algae, moving water is of great importance [63, 64, 65, 46, 48]. Waves and currents are accompanied by the turbulent motion of the water, which contributes to the absorption of biogenic elements by algae [66]. During our observations, we noted a high attachment capacity of zoospores. They detached from the glass only after being pressed with a dissecting needle. This allowed us to increase the flow of water and aeration immediately to the maximum value. The constantly maximum rate of water circulation in the pools helps remove poorly attached spores, while the high flow velocity accelerates the germination of sporophytes [67].

Figure 3. Sizes of young sporophytes on strings placed at different distances from the falling water. Pools in the village of Glazkovka.

In 1985, destruction of sporophytes was found on the strings on the 20th day after the spore seeding. In some cases, cells at one side of sporophyte decreased in size and acquired a darker color (Figure 4A). Then, empty cells were observed among normal ones (Figure 4B). The sporophyte entirely changed color from brown to bright green, and then became orange. The diatoms, surrounding the sporophyte, also changed their color. Subsequently, sporophytes completely destroyed and turned into a jelly-like mass. There remained

only their silhouettes, edged by diatoms (Figure 4C). The disease affected multicellular sporophytes and did not involve zygotes and single-row sporophytes. The zygotes form a solid wall and contain a supply of nutrients, which allows them to survive unfavorable conditions [68]. Due to the mortality of only multicellular sporophytes, their average size decreased (Figure 5). In nature, lysis of developing spores in a less intensive than that observed in the laboratory, in slowly running seawater [69].

Before the disease was observed visually, a high concentration of nitrites in water was detected. In Kit Bay, nitrites reached 0.9–2.7 Mkg/L (Table 2); in the pools, their concentration was higher than 1110 µg/L (Figure 6). The increased level of nitrites was apparently related to the disease and destruction of sporophytes. Nitrites are formed as a result of destruction of organisms and turning of NH₃ into NO₂ by *Nitrozomonas* bacteria [70]. It is probable that nitrite monitoring in pools can become a rapid method for detecting the onset of a disease of sporophytes.

Figure 4. Appearance of the diseased and dead sporophytes in the pools in the village of Glazkovka: (A) onset of disease; (B) progression of disease; (C) destruction of sporophytes.

Figure 5. Rate of growth of young sporophytes. Pools in the village of Glazkovka.

Figure 6. Concentration of nitrate and nitrite salts in the seawater flowing out of the pools. Village of Glazkovka, 1985.

Table 2. Results of hydrochemical supervision on water surface in Kit Bay in 1985-1986.

Date	T°C	Salinity. psu	O ₂		pH _o Mkg/l	pH _b	Al _k	PO ₄ -P	Pcom	SiO ₃	NO ₂	NO ₃	NH ₄
			MI/l	%									
21.08.85	19.1	31.82	6.46	120.7	7.46	7.32	2.075	0	4.5	0	1	0	44
11.09.85	18.0	33.75	6.37	118.0	8.11	7.94	2.028	9	10.0	160	0.9	13	44
10.10.85	12.8	32.63	5.99	99.1	8.09	7.96	2.242	5	6.0	0	2.7	0.3	0
19.08.86	16.2	32.95	6.73	119.8	8.68	8.50	2.315	0	5.0	800	2.1	30	0
11.09.86	16.6	33.49	6.51	117.1	8.80	8.60	2.419	2	4.0	580	1.7	15	0
10.10.86	13.5	33.58	7.06	119.5	8.88	8.71	2.344	1	5.0	0	0.9	16	0

Figure 7. Variations in seawater temperature and light exposure in the pools. Village of Glazkovka.

In 1986, a stabilization of the temperature at about 10°C (Figure 7) delayed the onset of the disease of seedlings by five days as compared to that in 1985. One of the frames in 1985 was found to be constantly facing the surface, and, by early September, it had the largest sporophytes. This led us to believe that the illumination is not sufficient for sporophytes. After increasing the illumination by 2000 lx (from 6000 to 8000) (Figure 7) and lifting the frames closer to the surface, we could reduce the concentration of erythromycin (0.5 units/mL) needed to cure sporophytes by an order of magnitude as compared to the recommended one [53]. Every year, during the second half of September, we could decrease the water temperature in the pools to 11.0–10.5°C, which allowed us to increase the illuminance to 8000 lx and lift the frames closer to the surface. Probably as a result of this, as well as due to erythromycin at a concentration of 0.5 units/mL used in the first year (1985), we could achieve the 100% coverage of the frames by seedlings. This high rate of sporophytes' survival kept for three years. The positive effect of erythromycin and high illuminance on survival of sporophytes in the pools facilitates sterilization of large

volumes of water in case of industrial cultivation of seedlings.

In 1985–1988, seedlings could be grown only in September. In our southern neighbors, the sea water temperature in September is higher than 16°C and destroys sporophytes. Therefore, they do not need to accelerate the sporophyte growth in pools in August, and vitamins are excluded from the nutritional supplements introduced into the pools according to their technologies [46]. Off the coast of northern Primorsky Krai, the water temperature decreases to 16°C by late September (Table 2) and becomes comfortable for seedlings transferred into the sea. Therefore, in 1986, we introduced vitamins to the nutritional mixture E-S again and then found that they contributed to a 2-fold increase in the rate of sporophytes' growth in the pools (Figure 5). A large demand for vitamin B₁₂ was also found in other species of brown algae [71].

In October 1986 and 1987, due to the dry autumn and weak bloom of phytoplankton, the water in the sea had a high transparency, and seedlings on the frames exposed at a depth of 6 m for adaptation turned white and destroyed.

Their subsequent transfer to a 12-meter depth was too late and also did not save them. Among sporophytes on the strings woven into the rope in September, only those survived that had got in the shadow. However, sporophytes rooted normally on the strings, taken in October directly from the pools and transplanted onto the ropes. It means that preventing sporophytes from destruction probably requires an artificial decrease in illuminance in the sea. For instance, in 1985, seedlings did not die due to the fact that a lot of clay had been brought to the coast, and the waves dispersed it all over the Kit Bay. It is likely that introduction of mineral fertilizers into the water of the farm can also be an effective way to “shading” and increase in the rate of growth of Japanese kelp. According to the technique used in China, 2 tons of fertilizer containing the 5–10% solution of ammonium salts are introduced into the water near a farm with one-year-old Japanese kelp during the season [72].

3.2. The Results of the Experiment in 100-Liter Tanks

The cultivation of Japanese kelp sporophytes in 100-liter tanks showed that the optimal conditions for their growth occur in pasteurized water (tank no. 10). The conditions in the tank where the water is supplied in part from the pool (tank no. 6), as well as in tanks treated with antibiotics (tank no. 1), and pasteurized and treated with antibiotic (tank no. 2), were somewhat worse. The sporophytes in the tanks where the water was taken entirely from the pool (tanks nos. 5 and 7) were smallest. In tanks no. 3 and no. 8, in which a dozen of two-year-old Pacific mussels were kept constantly, sporophytes were among the best ones; in the bath no. 8, the nutritional supplement was introduced only once (Figure 8). The positive influence of mussels on the growth of brown algae has been known since long ago [73, 74, 49]. The effect of treatment of strings on the growth of sporophytes has also been found. In the tanks, where the strings had been fired (either after boiling or without boiling), sporophytes were more than twice as large as those on the strings, which had been just boiled or not boiled (237.2 and 111.5 μm , respectively).

Figure 8. Length of sporophytes in 10 tanks. Village of Posyet.

3.3. The Results of Farm-Based Cultivation of Japanese Kelp

There are no natural beds of Japanese kelp in China. This alga was brought there in the 1940s by Japanese occupants [75]. Therefore, Chinese waters are free of algae-eating organisms capable of mass destruction of young sporophytes. This becomes clearly evident in southern Possiet Bay: this alga does not occur in its shallow-water coves. Unlike Kit Bay (station 8, Figure 1) at the algal farm in Reid Pallada Bay (station 4, Figure 1) we found only one species of the order *Amphipoda*. As a result, farmers freely expose frames with seedlings in the sea for on-growing (adaptation). In Primorsky Krai, natural beds of Japanese kelp occur off the middle and northern part of the coast, where the consumers of this alga—amphipod crustaceans of the order *Amphipoda*—can also be found in abundance. Their number may reach 60 individuals per frame. This is one of the most important components of the benthos, and it often dominates the benthic communities [76]. Crustaceans feed on young sporophytes, and the high density of food makes facilitates their search. The process of transplantation of fragments onto the ropes lasts for about two months. During this time, even zygotes turn into sporophytes and are immediately consumed by amphipods. As a result, only about 30% of seedlings remain on frames. In Possiet Bay, 300 sporophytes per 1 cm of nylon string can be found the next year; in Kit bay, only 15 sporophytes per 400 m of string, which means that they are eaten almost completely. Bringing the frames back from to sea to the pools is impossible, as sporophytes have grown in size and die in the pools. If the frames with the seedlings are not hauled out of the sea, sporophytes, which have grown to 1 mm in length, get destroyed, but new ones develop from zygotes that slightly reduces the productivity of kelp. These features of our region led us to the idea of rejecting the “adaptation” of seedlings in the sea and conducting it in pools instead.

Thinning of seedlings by weaving fragments of strings in ropes decreases their consumption. It makes more difficult for amphipods to find food. The rate of algal growth in the sea increases; thalli become harder, and this also reduces their mortality. As a result, in 1986, we rejected “adaptation” of seedlings in the sea and kept the frames with seedlings were in pools throughout the transplantation process. To provide the optimal conditions for culturing the grown sporophytes, it was required to change the water in the pools more frequently, but the regular introduction of nutritional supplements was no longer necessary.

Among the Japanese kelp that overwintered on the farm, the best thalli were found on the ropes, into which in the autumn bundles of 3–4 algae had been woven (Figure 9–11). In March, thalli on these ropes reached a length of 2.5 m and an average wet weight of 127 g. The thalli on fragments woven into ropes with the intervals of 20 and 30 cm proved to be worse; however, by the middle of May, the thalli were longer than 2 m, and their weight was higher than 300 g (Figure 9, 11). The worst sporophytes were on the strings woven with the intervals of 15 cm. Thalli on these fragments shadowed each other and

slowed down the flow rate more significantly than those with 30-cm intervals. The algal farm in Kit Bay was covered with ice for two months in 1985/1986. Probably due to the poor water circulation, the top part of the ropes were overgrown by the algae *Scytosiphon lomentaria*. As a result, on some of the ropes the upper two bundles of algae completely died. The growth of algae near the surface could be reduced also by the shortage of nutrients in this horizon. In January, the concentration of nitrites at a depth of 1 m is sometimes lower than that at a 5-meter depth [48]. By the middle of June, all the survived thalli were longer than 2 m.

The number of thalli on our fragments reached 32, and the total number on the rope, in the case of weaving with the 30-

cm intervals, was 130; with 20-cm intervals, 195, which was much larger than that in Chinese algal farmers [44]. However, at this high density by July 10 (328 days of culturing), the average length of thalli reached 343 cm, and the average weight was 530 g (Figure 9, 11, 12). Therefore, in terms of 1 ha, the harvest of Japanese kelp with fragments woven in every 30 cm was 79,4 t; every 20 cm, 119,1 t. The best results for a farm can be achieved by breeding new sorts and supplementing nitrogen fertilizers [77, 78]. In Japan, the most optimum number of algae in bunches is 3–4 copies; the distance between the bunches, 30 cm; the spaces between the ropes of 2 m; and the optimal depth for spring and autumn cultivation, 0.5–1 m [48].

Figure 9. Rate of Japanese kelp's growth in length on the plantation in Kit Bay in 1986: (1) length of blades on strings attached at 30-cm intervals; (2) length of blades on strings attached at 20-cm intervals; (3) length of blades in bunches attached every 15 cm; (4) length of blades in bunches, attached every 15 cm, in the autumn.

Figure 10. Rate of Japanese kelp's growth in width on the plantation in Kit Bay in 1986: (1) width of blades on strings attached at 30-cm intervals; (2) width of blades on strings attached at 20-cm intervals; (3) width of blades in bunches attached every 15 cm; (4) width of blades in bunches, attached every 15 cm, in the autumn.

Figure 11. Rate of Japanese kelp's growth in weight on the plantation in Kit Bay in 1986. (1) weight of blades on strings attached at 30-cm intervals; (2) weight of blades on strings attached at 20-cm intervals; (3) weight of blades in bunches attached every 15 cm; (4) weight of blades in bunches, attached every 15 cm, in the autumn. ingrown

Figure 12. Ingrown of Japanese kelp on the plantation in Kit Bay in 1986.

By beginning October 1986 the best of the thalli were at those ropes, in which in the autumn 1985 fragments woven in every 20 cm (damp weight of the thalli was 800 g, and dry - 160 g), that was 20% of biomass. By late October 1986, the size-weight parameters of the thalli planted in bundles (an average length of about 250 cm and a weight of 650 g) in autumn, as well as on fragments with the intervals of 30 cm from one another (a length of 220 cm and a weight of 430 g) were highest (Figure 9, 11). In them, the dry matter content was 12%, of which 45% were minerals, which indicates the suitability of the grown product for food. The worst of the algae was that removed from the fragments in spring 1986 and set on the ropes in bundles.

The algae most likely did not have time to acclimatize after the overwater operations in spring.

However, in spite of the good results of kelp cultivation at the farm, the obtained thalli poorly suited mechanical cutting due to their low rigidity. The machine could shred only the middle, hard part of the thallus, while its side parts had to be thrown away. In order to increase output of the marketable products, we set up an experiment on the further growth of the kelp at the farm. For this purpose, the entire experimental farm (1.5 ha) was left at sea for another year. The obtained results showed that the forcedly cultivated kelp cannot complete its lifecycle for the year in middle Primorsky Krai and does not die by fall. For the second year at sea, thalli greatly increase in weight and the length (Figure 13); as a result, the thallus grows harder, and the output of final product increases. By the following spring, the various schemes of seedling evened, and kelp reached the maximum productivity. By late May, thalli reached on average 270 cm in length and 1,200 g in weight, and until the middle of August, these values changed insignificantly (Figure 13). By late May, the average weight of alga on a one rope reached 156 kg. On one horizontal cable of 50 meters in length, there are 48 ropes, spaced at a distance of 50 cm. A total of 24 cables fit in one hectare; therefore, the yield per hectare reached 179,7 t wet weight or 29.6 t dry biomass. Prior to our experiments, the yield of two-year kelp per hectare in Primorsky Krai averaged at 70 t wet biomass [79]. Our modifications introduced in the technology of seedling cultivation—refusal from the transfer of frames in the sea—increased the survival rate of sporophytes, which resulted in a rise in the total yield of the farms in Kit Bay from 860 tons to 3200 tons. The excess of our seedlings was transported to the other two algal farms, where the yield also increased, which allowed them to collect a total of more than 5,000 t wet weight.

Figure 13. Rate of length, width, and weight growth of Japanese kelp on the plantation in Kit Bay in 1987.

In Possiet Bay, due to lack of herbivores, all sporophytes that overwintered on fragments of strings in 2006 survived, and the excessively high density (up to 300 ind. per 1 cm of string) resulted in their short length that did not exceed 40 cm. These sporophytes did not survive the summer heating and all died. Cultivation of Japanese kelp in the two-year cycle in Possiet Bay also has its own characteristic features.

Figure 14. Length, width, thickness, and weight growth of two varieties of Japanese kelp at station 4 in Posyet Bay: (A) growth of kelp blades in length; (B) growth of kelp blades in width; (C) growth of kelp blades in thickness; (D) growth of kelp blades in weight.

Good seedlings could be obtained only in the open Reid Pallada Bay (station 4, Figure 1), where the water flow is stronger than that in the semi-enclosed bays with Pecten farms (stations 1–2, Figure 1). However, transplantation of seedlings into the sea to new ropes has a negative impact on their survival. As a rule, all transplanted sporophytes died and survived either on rarefied or unprocessed ropes. This suggests the necessity of calculating the regional concentration of spore suspension that allows rejection of seedlings transplantation onto new ropes, and the importance of the fouling community that forms in the rhizoids.

The Japanese kelp populations located nearby may have different production capacities. Due to the low growth rate of the yearling kelp from the Gamov Peninsula (Figure 14), the bulk of the thalli destroyed during the summer warm-up. The two-year thalli grown to the marketable size was inferior to the kelp obtained from the maternal thalli from the Verkhovsky Islands in all respects, including weight (Figure 14). However, the 2-hectare storeyed farm installed for this kelp was not justified because it was located at the site sheltered from the prevailing currents (station 3, Figure 1). As in the other semi-enclosed bays, there were a lot of seedlings, but from of the fine sizes most of them died because of the warm summer.

The ropes with seedlings or spores are set in the sea in fall, when larvae of Pacific mussel have disappeared from the plankton. The following year, mussel larvae settle in rhizoids of kelp. By the time of kelp harvesting, mussel reaches one year age and cannot be used for food purposes. Most mussels on kelp harvested in Kit Bay are not bigger than 20 mm in length, and thus the ropes with kelp rhizoids and mussels living on them are discarded. The biomass of mussels with rhizoids at a 1-ha farm is more than 48 t. However, the rhizoids of dead or removed from the ropes kelp remain undestroyed over a year, and the mussels that settled on them do not fall off the substrate. If these ropes are left at the farm, the following year the mussels reach the commercial size. In Kit Bay, mussel reached 30.6 mm within 4 months after the kelp harvest, and in spring they became marketable with their shell length of 40–50 mm. In Possiet Bay, located more southerly, mussels reached 64 mm within 13 months after removal of the kelp. Thus, the use of algal plantations also for such a valuable food object as mussel significantly increases the profitability of marine farms.

As is known in aquaculture, only clean substrates must be used for optimum collection of shellfish larvae or algal spores. In the sea, substrates are cleaned by sea urchins, which scrape animals and algae off rock surface. Many of fouling organisms—calcareous algae, mollusks, bryozoans, and sea squirts—have a long life span, and if not removed from the substrate (the secondary succession is not created), they are able to occupy the entire bottom, and algal zoospores will not find a substrate for settling. In 1992 on ours observation about the closed bays ensuring shelter for the divers, the stocks of sea urchin *Strongylocentrotus intermedius* came to zero. Therefore, due to the large-scale catch of sea urchins in Russia since the early 1990s, fouling organisms that settled on rocks and uneaten rhizoids of destroyed kelp prevent spores from settling. This has resulted in a significant decline in the stock of Japanese kelp in nature [57]. The “Isoyake” phenomenon—occupation of substrates by calcareous algae—was first mentioned in Japan, because the sea urchin fishing there began much earlier than in Russia. Therefore, to restore the previous stock of kelp, the stocks of sea urchins should be rather restored than depleted, as a low density of breeding individuals reduces their natural reproduction rate. The good settling of sea urchin larvae onto Pecten collectors [80] allows us to recommend this method as the most affordable and effective. While being on these collectors, sea urchins feed on juvenile clams that helps them reach the viable stage without losses.

Catches of Japanese kelp cannot be increased to the previous high level without artificial reproduction. In the current economic situation, one of the methods of breeding this alga can be that offered by us in 1984: breeding of Japanese kelp on a horizontal spore-seeded cable fixed by cargoes on the bottom. To make the technological operations easier, we proposed spore-seeding of these cables in the hold of the vessel to be used for aquaculture. After settling of spores, the horizontal cable is hauled down to the bottom of

the sea directly from the hold. After the kelp reaches the marketable size, the cable is returned aboard using lifting mechanisms, and while it is being hauled up, the grown thalli are collected from it. The presented materials, in our opinion, can facilitate the process of cultivation and increase yields of the valuable alga and mussel.

4. Conclusion

The present publication demonstrates the possibility to grow-out the valuable kelp *S. japonica* to market size on algal plantations in Primorsky Krai, Russia, within one year. In order to ensure stable yield, a significant upgrade of the facility for seedling production is necessary: the water inlet and outlet of the pools should be easily maintainable; a more powerful refrigeration system, capable of lowering the water temperature in the pools to 10°C in summer, is required; the water flowing into the pools should be sterilized with UV lamps, and the pools should be illuminated by low-temperature fluorescent lamps. Decrease in temperature of sea water to 10.5°C and increase in its light exposure to 8000 lux, and also use of erythromycin for destruction of Gram-negative bacteria allowed us to increase the survival of young sporophytes in the pools. Even a single facility for growing seedlings of this alga can increase production at several algal farms where seedlings are used. In waters with no natural algal beds and, accordingly, no phytophagous organisms foraging on early sporophytes and reducing the density of bushes, the product can only be obtained in open waters with strong hydrodynamics, which promote a high rate of growth. The different genetic modifications of this alga manifest different production potential when grown on the same plantation. It is likely that Pacific mussel living on rhizoids of algae is a kind of nitrogen-fixing organism that stimulates their growth. Their combined cultivation can increase profitability of marine plantations.

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