Experimental larval rearing of the Japanese sea cucumber (Apostichopus japonicus) in Severnaya bay (Slavyansky bay, Sea of Japan)

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Abstract: Experimental larval rearing of the Japanese sea cucumber in a mini plant in Severnaya bay (Slavyansky bay, Sea of Japan) was carried out. The experiment did not reveal any relevant difference in time or speed of reaching different larval development stages or survival rate of the sea cucumber either with the diet supplemented with pigmented yeast Rhodotorula benthica or when fed with the Chaetoceros muelleri and Dunaliella salina phytoplankton. In the lack of the traditional phytoplankton-based fodder, it appears relevant and feasible for the aquaculture enterprises to use such fodder replacement options as Rhodotorula benthica marine pigmented yeast, a non-pathological species with low production cost that does not require any sophisticated cultivation techniques, easy to use and generally yielding a highly predictable production result.

1. Introduction
Japanese sea cucumber (Apostichopus japonicus) is one of the most expensive and sought for products on the Asian market [1]. The capture level of the commercial sea cucumber exceeds that of any other species harvested in the Primorsky Territory. The PRC still remains the main consumer of the Echinoderms, but the Japanese sea cucumber is the most preferred of all due to its higher quality and nutritional value.

An efficient way of reinforcing the natural accumulation of holothuria and the commercial product output is the development and implementation of the juveniles rearing technologies at the industrial facilities.

By the present moment, a number of recommendations, methodological guidelines and industrial standards intended to improve the growth indexes and survival rates of the Japanese sea cucumber larvae and juveniles [2-5]. Moreover, industrial water supply and life support system sanitation methods have been developed to prevent the massive death of the reared species at any stage of its growth, from reproducing adults to viable juveniles.

Regardless of all the recommendations and standards developed for all stages of the technical process, the problems of selecting a more stable fodder base (diet) for the larvae and settled juveniles remain unsolved; the diseases that occur at any development stage and the uncontrollable biotic factors of the media still can cause the death of the entire reared colony of animals.

The natural sea cucumber larva diet relies upon the unicellular phytoplankton, such as Dunaliella salina, Chaetoceros muelleri. The main problem for the phytoplankton cultivation is the infections that
may cause the death of the entire fodder culture colony. In the lack of phytoplankton, an alternative fodder of bacteria can be used [2].

When the settled larvae transform into juveniles (achieve the size of 2 mm), they need to be fed with finely ground fresh seaweed or dry plant powder soaked in sea water and filtered. The main unsolved rearing task is the continuous presence of high-quality artificial fodder of the required type. The present production problems can be solved by studying and introducing alternative fodder types for the Japanese sea cucumber larvae and juveniles with due regard to the production specificity and the geographical location.

The objective of the present study is to research the impact the marine yeast on the growth, development and survival rate of the Japanese sea cucumber reared at a mini plant located in Severnaya bay (Slavyansky bay, the Sea of Japan).

2. Research materials and methods

The potential breeding stock of the Japanese sea cucumber for spawning in the reading mini plant was collected by divers in the Severnaya bay water area (Slavyansky bay, the Sea of Japan).

Severnaya bay lies in the southwest part of Amur bay (figure 1). It is protruded into the continent in a quite prominent way, separated from Amur bay with Cape Maltsev and the southwestern edge of the Yankovsky peninsula. Coordinates: 131° 24' E; 42° 54' N [2].

![Figure 1. Severnaya bay (Slavyansky bay, the Sea of Japan)](image)

The main selection criteria were the weight of the animals of min. 150 g and the absence of body trauma. After the transportation of the animals and placing them into the spawning vessels, the biological analysis of 300 individuals was carried out by gonadal index calculation, smear collection and detection of the reproductive maturity degree. The average weight of one individual was 137 g, gonadal index 14.3%, average female fertility 1019.2 thousand cells.

The reproductive animals were held in the spawning vessels under the industrially controlled environmental parameters. The population density in the vessels did not exceed 0.1 individual per liter.
For better maturing, the water in the spawning vessels was increased by 0.5°C on a daily basis. The water circulation ensured the replacement of two volumes of water per day, the combined fodder input was 0.2 g per individual, O₂ saturation level of 80-100%, minimum water salinity value was 25% as per the guidelines [2, 3, 5, 6].

As the temperature in the vessels reached 18 °C, the spawning process was stimulated with a temperature method. The temperature was gradually increased by 0.5 °C to 23-25 °C, which stimulated the release of the reproductive products of the animals into the water. Due to the stimulation, the male spawning was more massive (50-100% of animals), thereby stimulating the females as well.

If the temperature stimulation method did not cause any positive result, the contrast stimulation method ("drying") was applied. The reproductive animals were held in the open air for 60 minutes, then rinsed with cool sea water and placed in the spawning vessels with the water temperature they have previously been. The individuals that did not start spawning after 2-3 stimulations were rejected.

The gender proportion in the spawning vessels was 1:3 (1 female to 3 males). Due to the absence of visible sexual dimorphism, the proportion of the individuals was regulated at the moment of release of the reproductive product (males: white, creamy, females: orange, bright pink) by moving the individuals into different vessels.

The fertilized eggs were placed in tanks for embryo incubation and rearing the larvae. The density of caviar placement was 2 pcs per ml, calculated with the MBS-10 microscope under the 2-4x magnification, Bogorov counting tray (colony count), a pipette and a glass. To prevent hypoxia, the water was mechanically stirred every two hours.

Every day at the same time samples from the larvae tank were taken with a glass tube from the surface to the bottom in several different spots of the tank. In every sample, on Bogorov counting tray under a binocular microscope, at least three portions of 10 ml were examined. The objective of examination was to detect the presence and number of any abnormal forms. When their share exceeded 25%, the batch was rejected (according to the guidelines) [2].

The larvae were reared in sea water filtered through 10-20 μm mesh filter; the water temperature was maintained at the level of 21-22° C with the minimum salinity value of 25%. At the dipleurula stage, *Dunaliella Salina* and *Chaetoceros muelleri* phytoplankton at the proportion of 1:1 were introduced into the larvae tanks. The amount of the introduced feeding suspension was determined as 10,000 cells/ml for the larvae population density of 1 individual per ml. The larvae were fed 2-4 times per 24 hours at one and the same time, before the replacement of water. In the absence of the required amount of phytoplankton, bakery yeast or marine yeast with the maximum cell size of 10 μm can be used [2]. Unlike the traditional bakery and brewing yeasts, the pigmented yeast *Rhodotorula benthica* contain high concentrated astaxanthin and carotene that improve the immunity and survival rate of the sea cucumber both at the larva stages and after settling [5].

After the larvae begin exogenous nutrition, in eight tanks the pigmented yeast (*Rhodotorula benthica*) were introduced. In the control tank (No.9), the *Chaetoceros muelleri* and *Dunaliella Salina* phytoplankton at the proportion of 1:1 were used (table 1). During the experiment, two times a day at the same time the portion of feed at the proportion of 10 thousand cells per ml (in the standard larvae population density of 1 individual per ml) was introduced, the population of the animals was counted, 50% of the water volume was replaced, the water was aerated, and the temperature and salinity values were measured. The larvae tank water was replaced before feeding to prevent washing out the feed.

**Table 1.** Average larvae population density and the input feed amount.

| Tank No. | Population density (individuals/ml) | Rhodotorula benthica (cells/ml) | Chaetoceros muelleri, Dunaliella salina (cells/ml) |
|----------|-----------------------------------|---------------------------------|-----------------------------------------------|
| 1        | 2                                 | 20,000                          | -                                             |
| 2        | 4                                 | 40,000                          | -                                             |
| 3        | 1                                 | 10,000                          | -                                             |
| 4        | 3                                 | 30,000                          | -                                             |
After the larvae reached the doliolaria stage, settling substrates with diatom algae on meshed PVC plates were installed in the tanks. As the larvae reached the doliolaria stage, meshed PVC substrates with the mesh size 1 mm were installed in the rearing tanks to increase the potential juvenile settling area. The number of plates in one cluster was calculated based on the population density of 500 individuals/m². As the substrates need to get coated with a layer of the attached diatom macroalgae for the animals to settle, the tanks with pentactula stage larvae were introduced homogenized *Sargassum pallidum*, *Sargassum miyabei* solution filtered through gas filter with the mesh size of 100 μm.

After that, the settled juveniles were fed with red-pigmented yeast (*Rhodotorula benthica*) and the *Sargassum homogenate*. As the size of the animals exceeded 0.5 cm, Chinese combined feed of 5-10% of the juvenile mass per 24 hours was introduced into the diet. The feed was input regularly, three times a day, after the tank water has been replaced. The amount of feed was regulated as it was consumed from the plate surface [1]. As the juvenile animals reached the size of over 1 cm with distinctive pigmentation of the skin, the tank water supply was switched to circulating supply. The water medium parameters were controlled on a daily basis. The salinity value was maintained at the level of 25% or higher, and the temperature of 15°C or higher.

### 3. Research results

The reproductive individuals of Japanese sea cucumber captured in Severnaya bay were adjusted to the industrial medium, and then 18 spawning sessions fertilizing the average amount of 73,380,000 mature eggs were carried out. The number of reproductive maturing facilitating degree-days was 1875 days. After the embryo stage was complete, on the 3rd day of life the first larva stage, early auricularia began, lasting for 4-6 days. On the 8th day, the majority of the larvae transitioned to the medium auricularia stage. On the 12th day of the larval development, the individuals transitioned to the final auricularia stage referred to as late auricularia. 15 days after egg fertilization, the larvae transitioned to the doliolaria stage. The stage duration was 2-3 days. On the 18th day, upon the transition to the pentactula stage, the larvae settled on the prearranged substrates. The stage lasted for 1-2 days. On the 20th day, the first settled juvenile individuals were found in the tanks (figure 2).

![Japanese sea cucumber larva development stage duration](https://example.com/larval-development.png)

**Figure 2.** Japanese sea cucumber larva development stage duration.
No difference in the time or speed of larvae settling was observed between those fed with the phytoplankton or bacterial feed.

In the period of the sea cucumber larvae survival rate observation, the introduction of *Rhodotorula benthica* yeast at the early, medium, and late auricularia stages was associated with the survival rate of 100%. Similarly, the 100% survival rate was found in the control tank where *Chaetoceros muelleri* and *Dunaliella salina* phytoplankton were introduced (figure 3).

After the transition to the doliolaria stage, the average population density in all the tanks reduced due to the complexity of the metamorphosis from auricularia to doliolaria (table 2).

**Table 2.** Average population density of the Japanese sea cucumber larvae at different development stages.

| Development stage       | Average population density individuals/ml; individuals/cm² |
|-------------------------|------------------------------------------------------------|
|                         | No.1 | No.2 | No.3 | No.4 | No.5 | No.6 | No.7 | No.8 | No.9 |
| early-late auricularia  | 5    | 4    | 9    | 11   | 6    | 15   | 4    | 17   | 6    |
| deliolaria              | 2    | 1    | 2.5  | 9    | 3    | 2    | 5    | 6    | 4    |
| pentactula              | 1.5  | 1    | 2.5  | 5    | 2.5  | 3.5  | 3.5  | 4    | 2    |

The survival rate of the larvae at different development stages in the experimental tanks varied from 13 to 100% (figure 3). In the control pool where the phytoplankton was introduced, the survival rate reached 66% (figure 3).

After the transfer to the pentactula stage, the survival rate in the tanks with red yeast used as a feed changed from 23 to 87%. In tank No.9K, where phytoplankton was introduced, the survival rate constituted 33% (figure 3). A possible reason of the low larvae survival rate was the immaturity of the reproduction substances which slowed down the development and the speed of metamorphosis to a settled individual stage. The greatest mortality rate was found at the larvae transition to the doliolaria stage, which is likely to be caused by the complexity of the metamorphosis process.

![Figure 3. Survival rate of the Japanese sea cucumber larvae at different development stages.](image-url)
In the Echinoderms breeding technology, the feed quality is the underlying factor influencing the growth and survival rate of the larvae and juveniles, so the optimal diet and feed selection is of greatest importance. Traditionally, the natural sea cucumber larva diet relies upon the unicellular phytoplankton, such as *Dunaliella salina*, *Chaetoceros muelleri*. Providing the basic phytoplankton-based diet for the larvae and juveniles of the Echinoderms bred in the artificial media is associated with a number of negative specificities including infections transmitted by these microorganisms, causing the death of the entire stock of the feed, relatively sophisticated technology of phytoplankton reproduction in artificial media, as well as the season-dependence of the said processes.

To breed one million viable juvenile individuals, over 2,000 liters of phytoplankton suspension of each species need to be cultivated. According to the practice, the main reason for the death of phytoplankton colonies are alien microorganisms that begin actively reproducing as the bay water temperature increases.

At the present moment, yeast is recognized as the most acceptable phytoplankton substitute, as it does not require any special cultivation techniques and are easy to use. In China, both fresh-dried marine yeasts and yeast suspension of *Rhodotorula* and *Debaryomyces* genera are being actively used as a feed supplement for the bred aquatic organisms [7]. Unlike the traditional bakery and brewing yeasts, the pigmented yeast *Rhodotorula benthica* contain high concentrated astaxanthin and 3-carotene that improve the immunity and survival rate of the sea cucumber both at the larva stages and after settling [7].

The experiment carried out at the Scientific and Production Mariculture Department of Severnaya bay, did not reveal any relevant difference in time or speed of reaching certain larval development stages or survival rate of the Japanese sea cucumber either with the diet supplemented with pigmented yeast *Rhodotorula benthica* or when fed with the *Chaetoceros muelleri* and *Dunaliella salina* phytoplankton species. In the lack of the traditional phytoplankton-based feed stock, it appears feasible and rational for the aquatic culture plants to use probiotic supplements consisting of live organisms and (or) microbial substances, stimulating the immune system, the digestion processes, the reproduction of the natural microflora of the animals.

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