

TRAINING MANUAL ON BREEDING AND CULTURE OF SCALLOP AND SEA CUCUMBER IN CHINA

[Contents](#)

**Prepared for the
Scallop and Sea Cucumber Breeding
and Culture Training Course
conducted by the
Yellow Sea Fisheries Research Institute in Qingdao
People's Republic of China
and organized by the
Regional Seafarming Development and
Demonstration Project (RAS/90/002)**

May 1991

Training Manual 9

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PART I

SCALLOP CULTURE IN CHINA

CHAPTER I

STRUCTURE AND BIOLOGY OF SCALLOPS

There are more than 300 species of scallop that have been identified worldwide. Among the 40 species distributed along the coast of China, Chlamys farreri, Argopecten irradians, Pactinopecten yessoensis and Chlamys nobilis are the most important economic scallops being cultivated in the country.

1.1 Geographical Distribution

Scallops are distributed world-wide. However, various species require specific environmental conditions for survival and reproduction and are therefore found only in coasts of certain geographical regions.

1. Chlamys farreri is a species native to the coasts of Northern China and western Korea. The suitable habitat for this scallop is where the water depth is 10–30 m, tidal current is swift, water temperature is relatively low, the bottom is rocky reef and the salinity and transparency are relatively high.
2. Chlamys nobilis is mainly distributed in Japan, Southern China and Indonesia. The environmental conditions of its habitat are quite similar to those of Chlamys farreri, except for water temperature, the suitable level of which for the former being higher.
3. Pactinopecten yessoensis occurs only in Japan. Through transplantation, it has been popularly cultivated in China for many years. Being a cold water species, P. yessoensis can only be cultivated in the temperate sea regions of Northern China.
4. Argopecten irradians, the bay scallop, has a wide geographical distribution along the Atlantic and Gulf coasts of the United States. After being transplanted into China in 1982, the bay scallop gradually became one of the dominant species of scallops cultivated in China.

1.2 Shape

1.2.1 Chlamys farreri

The shell of this species is medium in size and fan-shaped. Both shells are convex, but the right one is flatter than the left. The height is slightly greater than the length, while the width is $\frac{1}{2}$ of the height. The straight hinge is composed of umbo and an anterior ear and a posterior ear in each shell. The anterior ear is bigger than the posterior one. The shape of anterior ears in both left and right shells is different. The sunken ventral portion of the anterior ear of the right shell makes it possible to leave an aperture when the two shells are closed. The aperture is the only exit for byssus, which keeps the scallop firmly attached to a hard substratum.

The color of outer side of shell is variable, it being dark brown, light yellow, apricot pink or grayish white. There are about 10 (8–13) well developed ribs on the left shell and about 30 relatively thin ribs on the right shell, with many sharp brambles extending from the ribs on both shells.

The inner side is generally white with brown spots. However, the colour is pink in some individuals.

1.2.2 Argopecten irradians (bay scallop)

Though slightly smaller in size, both shells are more convex than those of Chlamys farreri. The outer side of the shell is yellowish-brown, with about 20 high brambles and rather wide ribs. The anterior ear is bigger than the posterior.

1.2.3 Pactinopecten yessoensis

This is bigger than other species, its shell being more than 20 cm long. The left shell is flatter than the right. The right shell is yellowish-white in colour, while the left shell purplish-brown. The anterior and posterior ears are same in size. There are about 23 (21–24) ribs on each shell.

1.2.4 Chlamys nobilis

The height of the shell is almost equal to its length. The colour of the outer side of shell is variable, it being light purplish-brown, yellowish-brown or pink. There are about 23 ribs on each shell.

1.3 Inner Structure

The description of digestive and reproductive systems given below is based on Chlamys farreri (Fig. 1).

1.3.1 Digestive System

The digestive system is mainly composed of mouth, labellum, stomach, descending and ascending intestine and anus.

- a. Labellum. It is situated at the end of gill and is composed of two pairs of labial palps, with one pair on each side. Each pair of labial palps can be divided into inner and outer labial palps. There are a lot of cilia on the inner side of each labial palp. The food is sent toward the mouth by means of movements of the cilia. Another function of the labial palps is to prevent the food from going back.
- b. Mouth. Looking like a split in the centre of two labial palps, the mouth is connected to the oesophagus, which is about 1 cm long and 3–4 mm in diameter.
- c. Stomach. Surrounded by the digestive gland, the flat oval-shaped stomach is connected to the oesophagus at its upper end and to the intestine at its lower end.
- d. Intestine. The intestine consists of the descending intestine, ascending intestine and rectum.

The descending intestine is located on the left side of the body at the ventral end. From the ventral position of the stomach, the descending intestine runs downward through the gonad and then turns upward along the lower end of the gonad to form the ascending intestine. There is a cream-colored crystalline style located in the cavity of the descending intestine. Its real function has not been clearly proved, but there are several conjectures, such as that it is an appendage of the reproductive organ, a part of the reduced tongue, helps in digestion, adjusts the digestion rate and stores food.

The ascending intestine initially goes upward along the back inner edge of the gonad, continues upward along the inner side of gonad until it enters into the digestive gland (digestive diverticula). The ascending intestine is connected to the rectum at the back of the soft part.

The rectum passes through the ventricle and goes downward along the back of the adductor muscle until it makes a 'U' turn to form the anus.

1.3.2 Reproductive System

Among the four species, only the bay scallop is hermaphrodite. The gonad is usually located between the postero-ventral part of the foot and the front side of adductor muscle and is crescent shaped. When the gonads mature, the cream coloured testis and bright orange ovary can be easily distinguished by naked eye.

The sperms and eggs are discharged out of the body through the breeding holes of kidney gland, which is situated in the region near the gonad.

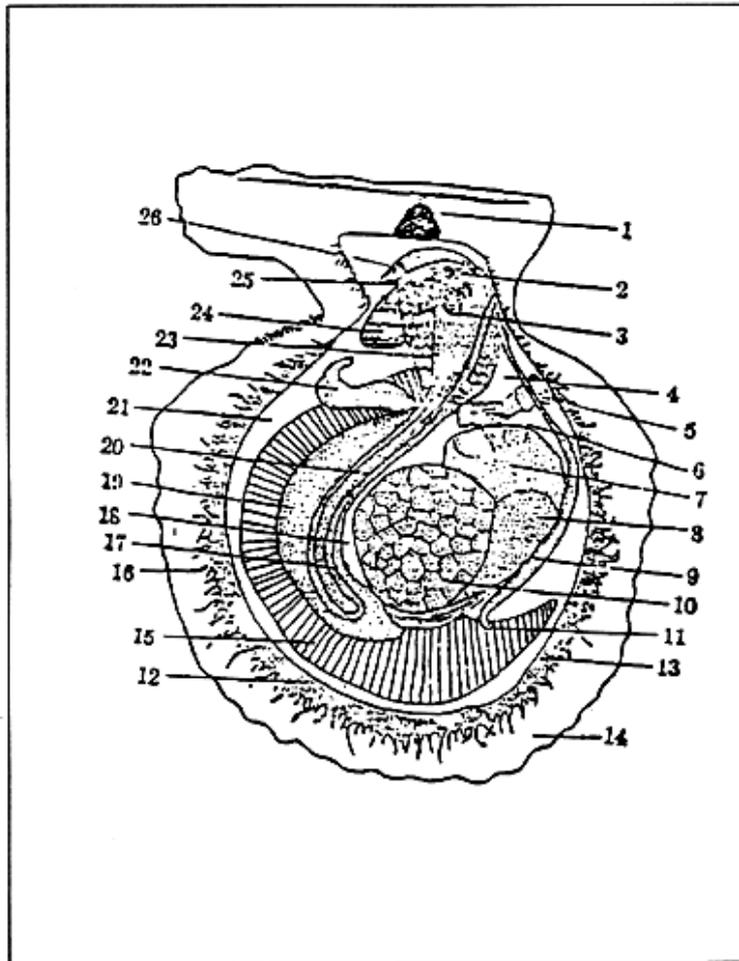


Figure 1. Internal structure of *C. farreri*. (1. ligament; 2. oesophagus; 3. stomach; 4. precordial cavity; 5. ventricle; 6. heart-ear; 7-8-10. abductor muscle; 9. rectum; 10. anus; 12. eye-spot of mantle; 13-14. right shell; 15. gill; 16. mantle; 17-18. kidney; 19. mantle cavity; 20. gonad; 21. intestine; 22. foot; 23. crystalline style; 24. labial palps; 25. mouth; 26. labellum).

1.4 Living Habits

1.4.1 Feeding

As in the case of other bivalves, the feeding style is passive. The food is filtered by gills and then sent to mouth by the movement of labial palps.

The food ingested by scallops is composed of phytoplankton, zooplankton, bacteria and detritus. Among

the phytoplankton, diatoms constitute the major component.

The filtration rate of scallops varies with the alternation of day and night. Generally speaking, the filtration rate is faster at night than during daytime.

1.4.2 Movement

Under normal living conditions, the two shells of a feeding scallop are slightly open and the tentacles on the edge of mantle are extra-extended. Looking into the open shells, the black eye spots can be seen clearly on the mantle.

If the environmental conditions become unsuitable, the scallop is capable of cutting off its byssus and swimming to the ideal location by means of a water-jet generated by closing and opening of both shells. The scallop can swim faster than any other species of bivalves. When it finds an ideal place, it secretes a new byssus and attaches onto the substratum again. Since the right shell is somewhat bigger than the left one, the scallop usually positions its body in such way that the right shell is lower than the left.

The ability to secrete the byssus is determined by the size of the scallop and the water temperature. A 1983 study showed that in the size range 1.0–3.0 cm, the bigger scallops themselves faster than the smaller ones at a water temperature of 13.5 °C. The scallops cultivated in water of 19.5 °C attached themselves more quickly than those in 13.5 °C. After it has grown up, the bay scallop discard its byssus, while *C. farreri* retains it throughout its lifetime.

1.4.3 Reproductive Cycle

In the bay scallop, the gonad condition of bay scallop is classified into six stages. Stages I–III are immature, IV is mature, and V–VI are partially spent and spent conditions respectively. Stage I gonad is small and transparent, and the only reproductive tissues seen are narrow tubules with primary germ cells. In stage II, the gonad has increased in size and is translucent. In gross examination, testicular or spermary and ovarian regions cannot be distinguished. However, microscopic examination would reveal that a few follicles have developed spermatogonia and oogonia and the gonad became enlarged. In stage III, the bisexual nature of the gonad can be seen from the proximal white testis area and the distal pale orange ovarian portion. Spermatogonia increase in number and can be seen in clumps. A few spermatozoa are also seen, as also many half-grown oocytes with stalk and a large germinal vesicle.

In stage IV, the gonad increases considerably in volume and assumes a round form. It contains thickly packed follicles. The testicular and ovarian portions are cream and bright orange respectively. Microscopic examination shows free, active spermatozoa and mature pear-shaped oocytes. In the partially spent gonad condition (stage V), testis and ovary are differentiated by the pale white and orange colors of their respective regions. The gonads retain some residual mature germinal products. Empty spaces in the follicles of stage V gonad distinguish it from stage III gonad. The completely spent (stage VI) gonads are light brown in color, with no differentiation between testicular and ovarian regions. The gonads are shrunken and flaccid with empty follicles.

In Jiaozhou Bay, the bay scallop has two spawning peaks in a year. One occurs from late May to June and the other around September. The maturity of bay scallop can be advanced to March in early spring by conditioning with suitable water temperature and adequate food supply. This enables the raising of several batches of spats in a year in Northern China.

1.4.4 Spawning Behavior

The bay scallop, which is hermaphrodite, usually discharges its sperms before releasing the eggs, with a time lag of about 15–20 minutes. Simultaneous discharge of both types of gametes has not been observed so far in the laboratory or in commercial spat rearing establishment. Spawning usually occurs from 7 to 9 o'clock in the evening. A few individuals may spawn during daytime.

The average fecundity of bay scallop in a single spawning is about 0.5–0.6 million, although a mature gonad contains as much as 2–3 millions eggs.

1.4.5 Sperm and Egg

The eggs of the bay scallop are enclosed in a thin membrane, are pale orange in colour and range in diameter from 53–56 μ . Being of slightly higher density than the seawater, the eggs sink to the bottom of the container a little after spawning (Fig. 2).

Much smaller in size, the mature sperm swims actively in the seawater. The sperm of the bay scallop retains its fertilizing ability for 6 hours after discharge in waters of 16–19 °C.

1.4.6 Fertilization

Fertilization in case of bay scallop, as in bivalves, takes place in seawater. About 20 minutes after fertilization, the first polar body appears on the animal pole of the fertilized egg in water of 20–22 °C. The appearance of the second polar body follows after another 5 minutes. The appearance of polar body is usually used as the sign of fertilization in commercial spat rearing establishments.

1.4.7 Cleavage

About 40–50 minutes after fertilization, the fertilized egg begins to undergo cleavage in water of 20–23 °C. After a series of cleavages, the embryo gradually develops into a swimming ciliated larva, which eventually reaches the trochophore stage about 10 hrs after fertilization, depending on the original condition of the egg, culture method and water temperature.

1.4.8 D-shaped Larvae

During the later phase of the trochophore stage, the shell gland begins to excrete the shell. When the shell completely encloses the soft body, the larva has reached the early straight-hinge or D-shaped larva stage. From fertilization to this stage, a time of about 22 hrs is required in seawater of 20–23 °C. The larva in this stage is also known as veliger.

1.4.9 Umbo Larvae

When the umbo begins to appear at the hinge region, the larva reaches the umbo stage. During this stage, the development of some special internal organs take place. To start with, a pair of translucent round balance organs are formed at the back of the digestive gland in the central region of the shell. Some very small particles are seen moving inside both the balance organs.

An “eye” spot appears at the lower part of the balance organ when the larvae are approximately 180 μ long. In some individuals, this spot is inconspicuous and irregular at first, but after 1–3 days it becomes regular in shape (round) and conspicuous with brown color. The appearance of eye spot signals the end of the swimming stage and the approach of metamorphosis. This is an important for indicator for the culturists to introduce spat collectors should into the tanks for the settlement of larvae.

The appearance of foot and round-shaped eye spot and thickening of shell margin indicate the end of

umbo stage of veliger.

1.4.10 Metamorphosis

Metamorphosis, which is a gradual process, is preceded by a stage in which advanced larvae have both a functional velum and a foot, and alternately swim about and crawl on the bottom or some clean hard surfaced substratum. This stage, termed pediveliger, may last for several hours or even days depending on the conditions provided.

Initially, metamorphosis is characterized by the disappearance of the velum and retention of the functional foot. A definite narrow band then appears on the shell margin, which indicates the ending of prodissoconch and the beginning of dissoconch or post-larval shell.

The larvae in this stage are also called crawlers. During the early period of this stage, the larvae can stop crawling and swim again if the conditions are not suitable for their settlement. After a few days of crawling, the swimming ability of larvae is gradually reduced until the velum is completely reabsorbed and the settling is completed.

Once settled, organs such as foot, velum and eye spot degenerate, while the gill and adductor muscles develops quickly. The appearance of dissoconch indicates that larvae have already metamorphosed into juvenile scallops.

1.5 Effect of Environmental Factors on Larval Development

1.5.1 Temperature

Temperature is a very important factor for the growth of larvae, since the enzyme in the larvae requires a relatively stable temperature to maintain its activity. The various metabolic activities of the larvae, such as digestion, respiration, growth, etc., are influenced by temperature.

Experiments and practice show that the optimal temperature for rearing of bay scallop larvae ranges from 20–23 °C. If water temperature is below the optimum, the growth rate of larvae decreases and the rearing period gets prolonged. If the temperature is higher than optimum, most of the larvae sink to the bottom of tanks and die during the early veliger stage and a only few individuals grow up to metamorphosis.

1.5.2 Salinity

Under normal conditions, the osmotic pressures inside and outside the larva's body are balanced or equal. If there is any variation in salinity, the larvae must consume a certain amount of energy to adjust their inner osmotic pressure to reach a new equilibrium with sea water. Thus salinity can directly affect the growth and survival of larvae. If salinity goes beyond the normal tolerance range of the larvae, they may die due to non-equivalent osmosis. The optimal salinity for the larvae of bay scallop is 25 ± 1 ppt.

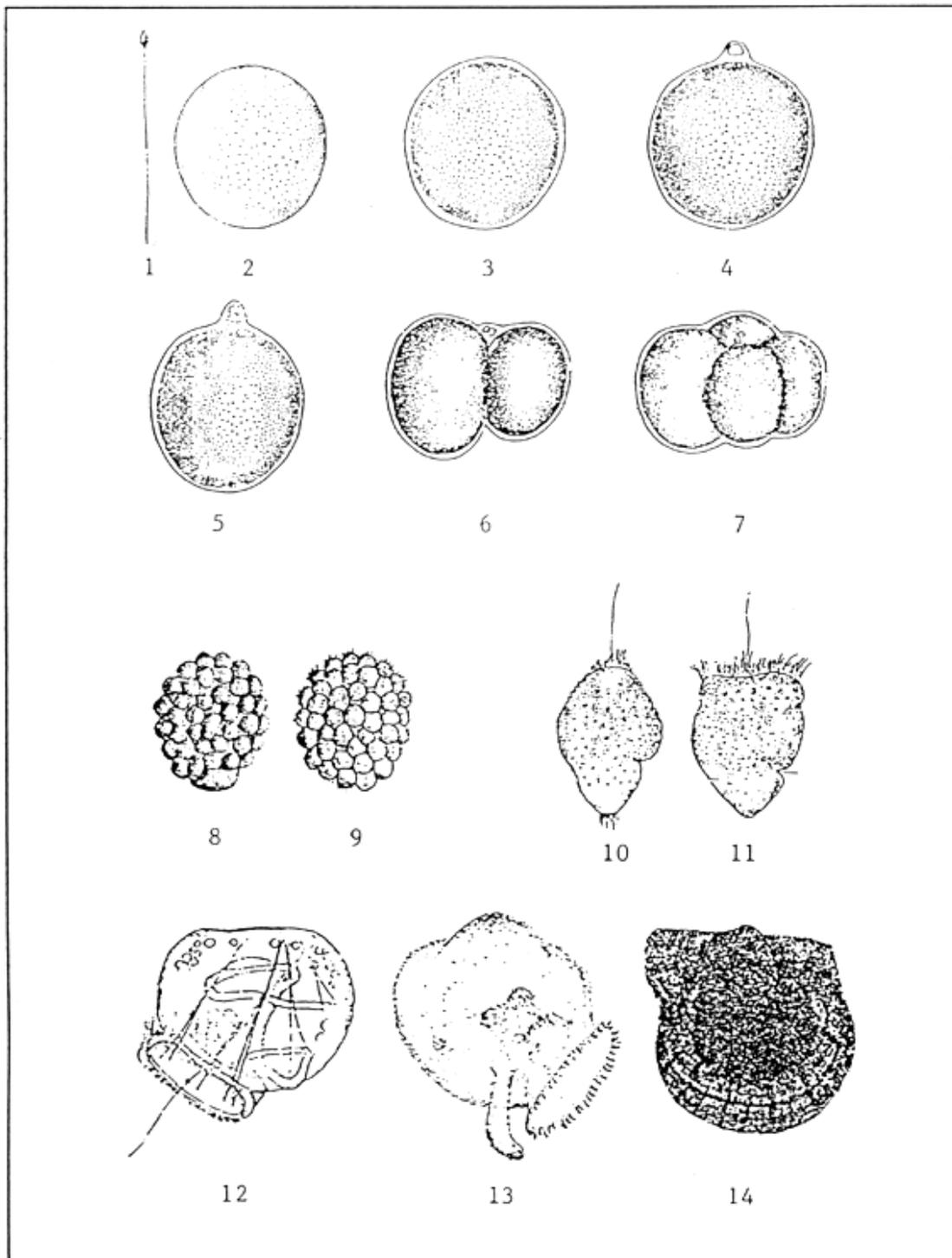


Figure 2. Developmental stages of the bay scallop, *Argopecten irradians*. (1. sperm; 2. egg; 3. fertilized egg; 4. extrusion of first polar body; 5. extrusion of second polar body; 6. first cleavage; 7. second cleavage; 8–9. morula stage; 10–11. trocophore stage; 12. veliger stage; 13. umbo larvae; 14. spat).

1.5.3 Water Quality

The optimal pH value for the larvae of bay scallop ranges from 7.8–8.2, while dissolved oxygen (DO) must be maintained at a concentration of not less than 5 ppm. The heavy metal content in seawater should be kept at the following concentrations:

| | | | | | |
|----|---------|-----|----|--------|-----|
| Hg | ≤ 0.004 | ppm | Cu | ≤ 0.01 | ppm |
| Cd | ≤ 0.03 | ppm | Zn | ≤ 0.1 | ppm |
| Pb | ≤ 0.1 | ppm | Al | ≤ 0.1 | ppm |

1.5.4 Rearing Density

The purpose of larval rearing is to produce as many larvae as possible in a limited water volume at minimum cost. Although the larvae can certainly grow quickly at a low larval density, the total number of juvenile scallops would be insufficient to meet the requirement of culture. On the other hand, over-crowding is undesirable, since it may reduce the growth rate of larvae and increase their susceptibility to diseases. Therefore, the determination of maximum concentration at which larvae can survive and the optimal concentration for their growth is an important consideration in the rearing of bay scallop larvae.

Under normal conditions, the optimum larval density in the case of bay scallop is 8–10 individuals per millilitre. If the conditions, such as food supply, water exchange, etc., are kept at the optimal level, the density may be increased to 20 individuals per millilitre. The rearing concentration should be adjusted according to the conditions provided in the hatchery.

1.5.5 Illumination

The effect of illumination on veliger larvae is significant. When they are exposed to intense illumination, the swimming larvae gradually sink to the bottom of the container due to their negative phototropism. If the illumination is too weak, the phytoplankton cultivated as food sinks to the bottom, which is harmful for the growth of larvae. Furthermore, a dark room is inconvenient for the hatchery operator. Based on observation and experiments, an illumination intensity ranging from 400–700 lux with diffused light has been found to be suitable for the growth of larvae.

1.5.6 Food

As soon as the digestive system is completely formed on the second day of D-shaped stage, the larvae can readily feed on unicellular phytoplankton. The naked flagellates, such as Monochrysis lutheri and Isochrysis galbana, are ideal food organisms, which can induce better growth than any organism having cell wall. Platymonas sp. and Chlorella sp. are also popularly used as feed for bay scallop larvae. Experiments have shown that mixed food composed of several species of phytoplankton promote more rapid growth than one-species food.

Artificial diet can also be used as larval feed, but it should be incorporated with unicellular phytoplankton. The optimal concentration of food cells varies with the size and developmental stage of larvae. The suitable concentration of food cells for the larvae of bay scallop is described in subsequent sections.

1.5.7 Antibiotics

Even under the best of conditions, there have been occasionally heavy mortalities of larvae and juveniles that could not be accounted for by mistakes in technique. In some cases, the heavy population of bacteria may cause epizootics and kill almost all the young bivalves in a short period of time.

Apart from treating the water as much as possible to keep it pure, antibiotics, such as terramycin, chloromycetin, penicillin, etc., are usually used to eliminate fungal infections in the larval culture tanks. The concentration of antibiotic depends on the degree of infection. In addition, if the concentration of heavy metals is higher than the permitted level, 3–6 ppm of EDTA should be kept in rearing tanks throughout the cultivation period.

1.5.8 Growth

In the temperate zone, the shell height of bay scallop increases at the rate of 1 cm every month during summer. When water temperature goes below 10 °C, the growth rate of shell gradually slows down, but does not stop unless the temperature drops below 5 °C. During early winter, the weight of the soft part begins to increase rapidly. In Northern China, the growth of shell of bay scallop stops during January - March.

1.5.9 Enemy Organisms

The enemy organisms of the bay scallop are usually divided into three groups, according to their living behavior.

- a. Predators. These include sea star, flounder, drilled conchs, etc. Among them, the sea star is the most harmful. These usually live in the gill cavity of the scallop and absorb nourishment from its soft part.
- b. Parasites. action also directly affects the filtration rate and thus impairs the growth of scallop due to lack of food. Pinnotheres is typical among such parasites. There are many species of sessile fouling organisms, such as mussels, sea squirts, sponge, diatoms, seaweeds, hydroids, oysters, flat worm, fungi, annelids, etc. Most attach themselves to the on-
- c. Foulers. growing facilities such as raft, rope and net cage and some attach directly onto the shell of scallop. These fouling organisms not only compete for space and food with scallop, but also block the mesh of net cages, affecting the growth and survival of scallops due to lack of food and poor water exchange.

1.5.10 Mortality

Mortality in the older culture sites is generally higher than in new seafarming areas, and occurs more frequently during summer and autumn than in winter and spring. Furthermore, heavier mortality occurs with higher culture density and bigger sized individuals.

An unsuitably high culture density may be the main cause of mortality. The reasons are: (1) the scallops cannot obtain sufficient food; (2) a greater amount of metabolic waste is produced, the decomposition of which will consume great quantity of dissolved oxygen; (3) the products of decomposition, especially sulphide, are toxic to scallops; some experiments have shown that when the sulphide concentration reaches 0.3 ppm, the filtration rate of scallop is reduced by about 30 %, and at 0.7 ppm, feeding and respiration stop; and (4) the weak individuals in a crowded and adverse environment are susceptible to disease.

Other important factors that cause mortality are high temperature and high density of fouling organisms.

The density of scallop culture should be determined based on the carrying capacity of the sea region and the technique of culture.

CHAPTER II HATCHERY EQUIPMENTS AND FACILITIES

The equipments required for the culture of bay scallop larvae include a water supply system, rearing tank, unicellular algal culture tank, heating system, aeration system, power system, observation room, laboratory, spat collector, etc. The design and construction of the facilities should depend on local conditions. The essential facilities and main equipments are described bellow.

2.1 Hatchery Site Selection

The hatchery must be situated in a pollution-free area with good quality seawater and mild waves. It should also be suitable for nursery culture. The hatchery should not be situated in an area with muddy bottom that contains high organic matter and water of low transparency.

Other factors that should be taken into consideration are availability of electricity, freshwater supply, good transportation facility and other supplies and services.

2.2 Installation Arrangement

The installation should be compact and rational, with the different facilities linked to each other. The observation room and the unicellular algae culture tanks should be near the hatchery workshop. The heating system should be concentrated, with the pipeline as short as possible to reduce the surface area for thermal diffusion. The foundation must be laid firmly to prevent land depression and tank leakage.

2.3 Water Supply System

The water supply system comprises the pump, pipeline, filter tank and purification tank.

2.3.1 Pump and Pipeline

Pumps are made of various materials, *viz.* iron, stainless steel, glass-fibre reinforced plastic, ceramic and plastic. The pumps that contain heavy metals or other harmful materials must be avoided. The pump house should be situated near the seashore to reduce the drawing distance. The drawing distance of pump should be greater than the drop between the pump house and the sea level and its lift should be greater than the drop between the top of the filter tank and the pump. In addition, the pump should be able to deliver the total water requirement of the hatchery in three hours at least. The inlet for the water supply should be placed where the water is deep and clear, and protected by cage. Two pumps should be available to ensure proper maintenance.

Wave-resistant wire-pipe or iron-pipe should be used as inlet pipe, since it extends into the sea. When iron-pipe is used, a drainage valve must be set to discharge the rusty water before transporting seawater to the filter tank. Polyvinyl chloride pipe is generally used for the indoor pipeline.

2.3.2 Precipitation Tank

The precipitation tank should be built on an elevated area or platform to save on electric energy. If it is built at floor level, a secondary lift facility should be used.

This tank should be divided into 2–4 chambers and its holding capacity should be 3–4 times that of the rearing tanks. Generally, the tank is 2 m deep, with a cover and an inlet pipe at the top. The bottom should have some slope and a sewer pipe with a valve. The tank's outlet pipe should be 30 cm above the bottom. The tank should be cleaned at least once a week when it is in operation.

2.3.3 Sand Filter

Sand filter is a mechanical filter device, which uses electrostatic force to absorb the suspended solids and separate them from the water.

The sand filter is placed in a concrete structure. Its top is almost at the same level as that of the bottom of the precipitation tank, but higher than the rearing tanks and unicellular algae culture tanks. If the precipitation tank, the sand filter and the rearing tank are on the same level, a secondary pump should be

installed for pressurized filtration. The total filter area (m²) should be about 1/30 – 1/40 to the total volume of rearing tank (m³). For the same filtration area, two filters will be better than a single filter for performance and maintenance.

To the top of the filter tank is fixed an inlet pipe, which comes from the precipitation tank. At the bottom, there is an outlet pipe to the rearing tanks in the hatchery workshop and a water storage space up to 15–20 cm in height, above which is placed a plastic or cement sieve plate of 1 cm mesh to support the pebble and sand filtering layers above. Sand and pebbles are filled in according to size in different layers, which are separated from one another by sieve plates. The composition of these filtering layers is given below:

| Layer (from the top) | Granular Diameter (mm) | Thickness of Layer (cm) |
|----------------------|------------------------|-------------------------|
| 1 | 0.1–0.2 | 15–30 |
| 2 | 0.2–0.3 | 20 |
| 3 | 0.4–0.5 | 10 |
| 4 | 1 – 2 | 5 |
| 5 | 5 – 10 | 5 |
| 6 | 20 – 30 | 5 |

When a filter is used for the first time, it should be filled in reverse with water entering from the base, to ensure its normal working. Then it can work normally. Filling water from the top will destroy the sand layer. The layer should not be allowed to dry up when the filtering stops, in order to avoid the rising air bubbles that would stir up the sand. A reverse washing should be resorted to every 3–4 days.

2.3.4 Rearing System

The rearing system includes a parent scallop-conditioning tank, spawning-cum-hatching tank, larval rearing tank all three of same size, preheating tank, aeration and inlet pipeline and heat exchangers. All of these are built in the hatchery. There is need for heat conservation and a light control device. An observation room should be provided at the side. Detailed specifications of these facilities are given below.

- a. Rearing tanks. The rearing tanks used for commercial production have different sizes. The small, medium and large rearing tanks have volumes of usually less than 10 m³, 10–30 m³ and 30–100 m³ respectively, with respective depths of 1.0–1.2 m, 1.3–1.5 m and 1.5–2 m. The tanks are generally rectangular with rounded corners. To facilitate easy handling, the tanks are sunk into the ground with only about 80 cm of their height above the. The inside surface of the tank should be smooth. The slope of the bottom is 0.5–1 %. At the bottom it has 1–2 outlets, each with a 5–10 cm long plastic pipe extending to the sewer channel. The discharge capacity should be such that all the water from the tank can be drained in half an hour.

The newly built concrete tank must be soaked with water for one month to remove the alkalinity, and the water should be changed every 3–5 days to keep the pH below 8.4.

- b. Inlet and outlet pipelines. Each tank should have at least one inlet valve. The main inlet pipe and its branches must have enough flow and should be able to fill all the tanks in about 8 hours. Below the outlet pipe, there is a sewer channel with a slope of 0.5–1.0 % to avoid water accumulation. The channel must be able to drain off the water without overflowing in case all the tanks are being drained simultaneously. It should be 80 cm in width and 60 cm in depth beneath the tank bottom and can be used for larva transfer from tank to tank. There are cover plates on the channel for easy routine work and walking.

2.3.5 Heating Equipment

Large scale larval rearing stations use boiler for heating. Filtered water is poured into the preheating tank and then steam produced by the boiler is let in to heat the water. A boiler with an evaporating capacity of 1 t/hr could meet the demands of a 400 m³ rearing volume.

The preheating tank should be near the boiler and its total carrying capacity should be about $\frac{1}{4}$ – • of the capacity of rearing tanks. The steam intake pipe, which extends vertically down to the lower part of the tank, has a control valve just before it enters the tank. A little before this valve, there is a branch pipe for draining purpose.

2.3.6 Aeration System

Roots blower, which has large airflow, steady pressure and cannot be easily polluted by oil, is suitable for large scale hatcheries. A blower with pressure of 0.2 kg/cm² will be suitable for a water depth of 1 m.

Polyvinyl chloride (PVC) pipe is used for aeration pipe, while a plastic flexible pipe with an airstone at the end is used for branch pipes. Airstones of 100 and 80 meshes can supply sufficient air to 4–5 m³ and 2–3 m³ water masses respectively.

2.3.7 Algal Culture Unit

Unicellular algae comprise the main food of cultured scallop. Their culture is, therefore, important for use in hatcheries.

Details of various components of the unit and their operation are furnished below.

a. Equipment and Materials

i. The algal culture room

The algal culture room should face South or North and there must be no high building around to block the sunlight and keep out the wind. The light intensity should reach 10,000 lux on sunny days, which is achieved by providing for light incursion from more than half of the total area of the roof and walls. For this purpose, corrugated tiles of glass fibre reinforced plastic are used in the roof, while doors and windows are provided on the walls. Curtains are used to regulate light intensity.

An aeration system should be installed, failing which aeration is done by regular manual agitation.

The water supply system is similar to that used for the larval rearing pond. But the water must be filtered by ceramic filter or disinfected with UV light or by a suitable chemical.

The capacity of the algal culture tank should be the same as that of the larval rearing tank.

The algal culture tank should be at a higher level than the larval rearing tank or it can be built upstairs, to facilitate easy transfer of algae to rearing tanks. However, the drainage pipeline systems of the two tanks must be separate, in order to prevent chemically polluted water of the algal culture tank from entering the larval rearing tank.

ii. Seed algae preservation room

Besides having the same equipments and requirements of the culture room, the preservation room requires both heating and cooling. The temperature should not be lower than 15 °C in winter, or higher than 25 °C in summer.

iii. The algal culture tank

The tank should be rectangular with rounded corners and laid out from South to North. The area of one pond is about 10 m², with a depth of 70 cm. There should also be some small ponds and deeper ponds. The smaller ponds can be used for seed algal culture and the deep ponds can be used for large scale algal culture to feed the broodstock. The deep ponds can also be used as larva rearing ponds.

The bottom and the walls of the tank should be covered with white ceramic tiles. The bottom should have a certain slope, so that the water can drain away completely by gravity flow. The inlet pipe and the algal transport pipe should be easy to clean.

If a new cement tank that has not been water-soaked needs to be urgently used, it must be painted with Rt waterproof coating.

b. Equipment Disinfection

The flasks and other glass containers should be washed, their mouths covered with a piece of paper and then sterilized in an oven for about 2 hours at 120°. The spoons are sterilized in alcohol and the emulsion pipe and PVC pipe with boiling water. Tweezers and other metal tools can be heated over a fire for disinfection.

The tanks are disinfected by one of the following methods:

i. Bleaching powder disinfection

The tank is covered with bleaching powder paste and then washed with water.

ii. Potassium permanganate disinfection

The tank is first washed with 25 ppm potassium permanganate solution, which is then diluted to 5 ppm and allowed to soak for 15 minutes. It is then drained and the tank washed with water.

iii. NaClO disinfection

The tank is cleaned and filled with water, to which is added the requisite quantity of sodium hypochloride (NaClO) to provide 3–5 ppm effective chlorine. After 4 hours, it is neutralized by the addition of sodium thiosulphate, which renders the water suitable for inoculation.

iv Phenol disinfection

The tank and the tools should be washed with 3–5 % phenol solution and soaked for an hour before washing with water.

Cupric sulphate and some other chemicals are also used for disinfection.

c. Culture of Stock Algae

i. Solid culture media

In the preparation of any solid medium, at first the required quantity of nutrient salts are added to a certain quantity of disinfected seawater. The nutrient salt composition differs for different algae. For example, in the case of *Platymonas* spp., the requirement is N:P:Fe = 10:0.1:0.1 ppm. Agar is then added at the rate of 1.5–2.0 % of the medium solution, which is heated till the introduced agar dissolves completely. The culture tubes should be blocked with cotton stopper and the culture dishes covered. All the tubes and dishes should be wrapped in paper, after which they are placed in an autoclave for 40 minutes disinfection. The tubes must be kept in a standing position. After the culture tubes and dishes have cooled down, they are inoculated with the stock algae and cultured under optimum light (100–500 lux) and temperature (10 °C) conditions. When the algal colony is established, the tubes and dishes are moved to the stock algae preservation room, where they can be stored for 6–12 months at 5–8 °C.

ii. Liquid culture media

For preparing liquid culture medium, flasks of 250–500 ml capacity are filled with filtered seawater and heated to boiling (90–100 °C). After the water cools down, requisite quantity of nutrition salts are added, followed by inoculation with the stock algae to be cultured. Culturing is done in suitable conditions of light and temperature. Change of bottles and addition of nutrition salts enhances the growth of the algae.

d. First-level Culture

10,000 ml capacity flasks are used for first-level culture. The flasks should be cleaned by water and disinfected in the autoclave. Requisite quantity of seawater is poured into the flasks and boiled water and nutrient salts are added after it has cooled down. It is then inoculated with the concerned alga taken from the pure culture. The flasks are covered with disinfected paper. Different kinds of algae should be laid separately. The flasks are kept under optimum illumination from an indirect light. The flasks should be shaken at least 3 times a day. For getting luxuriant growth, some extra quantity of culture medium is added to the extent of 1/5; – ¼ of the total volume of culture medium in the flask once every 2–3 days or even daily. If one alga is contaminated by another kind, it should not be used as stock alga any more, but can be used to feed the larvae. If the algal culture is contaminated by protozoans, it must be discarded outright.

e. Second-level Culture

Flasks and jars of 20,000 ml and small tanks of about 1 m³ capacity can all be used. The water used for flasks should be boiled, while the water used for jars and small tanks is treated similarly as in the case of third-level culture.

f. Third-level Culture

The third-level culture is the production culture. The tanks and the tools (such as mixer, air pipe, airstone) should be disinfected. The depth of culture medium (seawater + nutrient salts) in the tanks is about 10–20 cm. The inoculation volume is about 1/5; – • of the total volume, depending on the amount of the stock algae.

The tank should be aerated or stirred 4 times a day and extra culture medium added every 2–3 days. The light intensity must be regulated. Artificial light may be considered on cloudy days.

Within about 5 days of culture, the density of the alga can get to 2 million cells/ml. It can be readily used as larval food. Algae slightly contaminated with protozoans can be used, but cultures heavily infested with

protozoans must be discarded.

Feeding the larvae with several kinds of algae has been seen to be better than feeding with only one species.

g. Culture Conditions of Selected Unicellular Algae

The algae which are commonly used for feeding the larvae of the bay scallop and sea cucumber include Phaeodactylum tricornutum, Isochrysis galbana, Dicrateria zhenjiangensis, Platymonas spp. Their culture requirements are detailed below.

i. Phaeodactylum tricornutum Bohlin

- Temperature: 5–20 °C; optimum around 10 °C
- Nutrient salts: N:P:Fe = 20:1:0.1
- Light: 3,000–5,000 lux

ii. Isochrysis galbana Parke

- Temperature: 10–30 °C; optimum around 25 °C; the growth will be slow when temperature is under 20 °C
- Nutrition: N:P:Fe = 10:0.5:0.1 and some trace elements and 1 ppm vitamin B1 and B12
- Light: 3,000–8,000 lux

iii. Dicrateria zhanjiangensis Hu var. sp.

- Temperature: 18–28 °C; optimum: around 28 °C
- Nutrition: N:P:Fe = 20:0.1:0.1 with some trace elements and 1 ppm vitamin B1 and B12
- Light: 3,000–8,000 lux

iv. Platymonas spp.

- Temperature: 15–30 °C; optimum around 20 °C
- Nutrition: N:P:Fe = 20–50:1:0.1
- Light: 5,000–10,000 lux

h. Algal Count

There are many counting methods, but the haemocytometer method is most commonly used.

The haemocytometer is a glass plate, which is thinner than the commonly used glass slides. The central depressed part of the haemocytometer is divided into 9 squares, each measuring 1 mm² with a depth of 0.1 mm. When covered by a cover slip, each square has a space of 0.1 mm³.

For counting, a small amount of water containing the algae is put on the counter beneath the cover slip and the number of algae in 2 of the squares are counted under a microscope and their average taken. Two sub-samples are taken from every sample for counting.

The number of algal cells per ml of algal culture water is calculated as follows: average number per haemocytometer square × 10,000 × dilution factor.

i. Stock Solution of Nutrient Salts

Usually, NaNO₃ is used as nitrogenous fertilizer, KH₂PO₄ as phosphate fertilizer, FeC₆H₅O₇.xHO as

ferric fertilizer and Na_2SiO_3 as silicon fertilizer.

It is more convenient to use stock solutions in algal culture. The concentration of nutrients in the stock solution should be several folds that of the same nutrients in the seawater that is going to be used for algal culture.

CHAPTER III BREEDING AND LARVAL REARING

The account given below refers to the production of spat of the bay scallop, Argopecten irradians. The various steps include (1) cultivation of parent scallops, (2) induction of spawning, (3) fertilization, (4) rearing of larvae and (5) collection of spats.

3.1 Broodstock Culture

3.1.1 Biology

Being a hermaphrodite each individual bay scallop has both testis and ovary, as proximal and distal parts of the germinal gland. The ovarian and testicular parts are located along the outer margin and inner side respectively of the ventral region of soft parts. An immature gonad is usually covered by a black film. When the black film disappears, during the maturation period, the bisexual nature of the gonad can be made out from the cream coloured testis region and the orange coloured ovarian portion. In Northern China, the bay scallop has two spawning peaks in a year, the first in May and the second around September. In Southern China, the bay scallop spawns several times in the course of a year.

By culturing the scallops with adequate food in water of temperature maintained at optimum level, it is possible to advance their maturity from May to March in Northern China.

3.1.2 Conditioning

In the normal course, the bay scallop breeds in May and the resultant spats can be cultured only for about 6 months before the temperature drops down sharply after December. Since the size attained by then is less than the more preferred marketable size of 6 cm shell height, it is necessary to prolong the culture period. For this purpose, the breeders are cultivated in Northern China in indoor tanks, where the temperature can be maintained at the optimum level, which makes it possible to advance their maturity from May to March. The advancement of spawning by two months, by which time the scallops easily attain the marketable size. However, several factors are required to be taken care of in order to be able to bring the breeders to good conditions.

a. Selection of Breeders

Cultured scallops with 5–6 cm in shell height can be selected as breeders. Fouling organisms attached to the shells must be removed and cleaned by brushing as soon as the scallops are taken from their culture sites. Care should be taken while brushing so as not to damage the ligament. If the ligament is damaged, both shells of the scallop would never close as usual and the scallop soon dies. Well-selected parent scallops are usually put into lantern net cages and cultivated in the breeder tanks. The lantern net cages, tanks, etc., which could get in touch with scallops must be sterilized with potassium permanganate or any other disinfectant.

b. Breeders Culture Density

A density of 80–100 scallops per cubic meter has proved to be suitable. Each chamber of a lantern net cage can provide adequate space for 15 scallops. At this density of stocking, adequate fertilized eggs can be obtained for larval rearing in each tank.

The water in the breeder tanks should be totally renewed every day with pre-heated water. When the old water has been drained out completely, the sediment on the bottom of tanks must be removed and cleaned every day before the tanks are filled again. Scallops in the lantern net cages should be checked at least once a day for dead individuals. Dead scallops must be removed as soon as possible in order to prevent the rest from being infected with disease.

c. Water Quality Requirement

In order to keep the temperature as stable as possible, two or more tanks are used as the pre-heating tanks. With the constant availability of pre-heated water in the pre-heating tanks, the water in the broodstock tanks can be renewed any time so as not to cause temperature fluctuation. Two pre-heating tanks are usually used alternately.

The concentration of dissolved oxygen in the water of the broodstock tanks must be kept at more than 4 ppm and at pH 7.7 to 8.2.

d. Food

Unicellular algae, such as Phaeodactylum tricornutum, Isochysis galbana and Thalassiosira pseudonana, are effective food for parent scallops. In the early stage of broodstock rearing, P. tricornutum is the main food for parent scallops as it can be easily cultivated in low temperature. The other algae become the dominant food during the later stages of rearing.

The daily ration of food organisms for parent scallops is 20–80 l per m³ of breeder tank water, with the exact quantity depending on the size of cells and the feeding ability of the scallops. It is generally given in six equal installments. Experiments have also demonstrated that mixed feeding with several species of unicellular algae promote more rapid gonad development than feeding the same quantity of any one species alone.

Once a film appears on the water surface, the ration must be reduced as quickly as possible. The appearance of a film means that the food ration has gone far beyond the feeding ability of the scallops; pseudofaeces are produced to form a film on the water surface. Overfeeding is harmful and can cause heavy mortality. By adopting the steps described above, the parent scallops can be maintained at a survival rate of 70–80 %.

The artificial diet that has been formulated by the Yellow Sea Fisheries Research Institute has proved to be an effective food for parent scallops, even better than unicellular algae to a certain extent. Fed with this diet, the scallops can attain a gonadal index, which is 25 % higher than that obtained with unicellular algae, and have a better survival rate up to even 98 %. With such advantages as easy processing, low cost, rich nutrient content, this diet can completely replace the unicellular algae as food for parent scallops during breeder rearing.

Feeding the artificial diet at night and the unicellular algae in daytime is an effective and practical method for broodstock rearing.

e. Aeration

Among the shellfishes, bay scallop is one species that consumes a lot of oxygen for survival and growth. If DO concentration is lower than 4 ppm, scallops open their two shells much wider than normal and their behavior, such as feeding and moving, becomes sluggish. Thus aeration is a critical measure. Aeration can supplement enough DO for scallops.

Furthermore, aeration can help to check if spawning has occurred. In an aerated tank, a lot of bubbles would appear on the water surface as soon as the scallops begin spawning.

3.2 Spawning, Fertilization and Hatching

3.2.1 Observation of Gonad

As the date of spawning approaches, intensive checking and observation of gonads are required. Gonadal index is usually used to determine the development of gonad.

The formula for calculating gonadal index is : $G = (gw/sw) \times 100 \%$, where G is the gonadal index (%); gw is fresh weight of total gonad (g); and sw is fresh weight of total soft parts including the gonad (g).

When the average gonadal index reaches about 16 % and the black film on the surface of the gonad region disappears, it is to be understood that the scallops are about to spawn.

3.2.2 Checking on Spawning

As mentioned above, spawning can be easily noted according to the appearance of a number of bubbles on the water surface. If there is no aeration system in the tanks, a water sample should be taken from the bottom of the tank every 2 hours before the water is replaced during the later part of breeder rearing and the sample examined under a microscope for the presence of gametes. When the scallops are about to spawn, their food should be given at least two hours after the renewal of water. Once spawning occurs, feeding must be stopped and aeration increased. When the density of eggs reaches about 30 eggs per ml, the aeration must be stopped and the parent scallops taken away from the tanks.

3.2.3 Counting

Proper counting is a very important measure to estimate correctly the number of fertilized eggs and larvae. The counting helps in regulating the density of fertilized eggs in the tanks, in determining the quantity of food organisms required for the larvae and in determining the number of spat collectors required and the timing of their introduction into the tanks.

There are several methods for counting. The simplest but very effective counting method is described below.

A plastic or glass tube, with a diameter of 0.5 – 1.0 cm, is usually used as the sampling tube. The length of tube is about 20 cm more than the depth of the tank. A 1,000 ml beaker is kept ready for collecting the water sample.

Before sampling, the water in the sampling tank should be stirred by a swing board, so as to evenly distribute the eggs or larvae in the water column. As soon as stirring is stopped, the sampling tube is stuck vertically from the surface of water to the bottom of the tank. When the tube reaches the bottom, the hole on the upper end must be closed by the thumb and the tube lifted up as quickly as possible. Allow the sampled water in the tube to flow into the collecting beaker by loosening your thumb. Several samples should be taken from different points in one tank and all samples taken from one tank should be collected

into one beaker. The beaker must be aerated to evenly distribute the eggs or larvae in the sample, after which at least 3 samples are taken by quickly sucking out certain volumes of water from the beaker. The number of eggs or larvae in the samples are then counted in a haemocytometer under low power (10×4) microscope.

3.2.4 Hatching

Bay scallops being hermaphrodite, the ration of sperms and eggs is impossible to control. Therefore, the suitable density of fertilized eggs and normal development of larvae should be ensured by an adequate food supply and optimum environmental conditions. Experiments have shown that the fertilized eggs could successfully develop into the stage of D-shaped larvae if the density does not exceed 30 eggs per ml.

After the spawning is over, the water in the hatching tanks should be stirred with the swing boards for 30 minutes to prevent the eggs from sinking to the bottom.

i. Effect of salinity on hatching

The fertilized eggs can be normally hatched in a salinity range of 17–35 ppt, the suitable range being 22–33 ppt, with 27 ppt as the optimum salinity (Fig. 3). The salinity can be adjusted by addition of fresh water or sodium chloride, as necessary.

ii. Collection of D-shaped larvae

The fertilized eggs of bay scallop can develop into D-shaped (straight hinge-larvae) 22 hours after fertilization in water of 23 °C. Just like the larvae of other bivalves, the well-developed D-shaped larvae are usually found swimming in the upper layer of the water column. The D-shaped larvae can be collected by a small trawl net made of JP-120 sieve cloth with a mesh of 41 • and transferred from the hatching tanks to larval rearing tanks. By towing several times, most of the larvae swimming in the upper water layer can be collected and transferred to the rearing tanks. Fewer larvae swimming in the middle layer and bottom of the tanks can be collected by siphoning or draining the water out of the tanks.

3.3 Larval Cultivation

3.3.1 Density

The rearing density depends on the rearing technique, food supply, capacity of tanks and quality of larvae. It ranges from 5–15 larvae per ml.

3.3.2 Temperature

Experiments have shown that at higher temperatures within the desirable range a higher growth rate can be obtained. When grown in water of 16–21 °C, the first metamorphosed individual was noticed 12 days after fertilization. When grown in water of 22–23 °C and 27–25 °C, larvae reached settling stage in 10 days and 8 days respectively.

In Northern China, it has been shown that rearing larvae of bay scallop in 22–23 °C water temperature could shorten the rearing period and reduce costs and mortality. During the rearing period, the fluctuation of water temperature should not exceed ± 2 °C.

3.3.3 Illumination

The larvae of bay scallop can develop well into the juvenile stage under an illumination range of 300–800 lux, but they may hide in the corners or swim in the middle layer of tanks if exposed to more intense illumination. Aggregation of larvae may cause heavy mortality and therefore the illumination is usually controlled in the range of 300–500 lux. The larvae and food cells are all well-distributed in the rearing tanks under suitable illumination.

3.3.4 Aeration

Aeration is not imperative for larval rearing, especially at low density culture level. When larvae are reared in higher density, aeration should be provided to prevent the larvae from aggregating.

3.3.5 Salinity

The larvae of bay scallop can survive and grow in salinity range of 18–36 ppt; 23 ppt being most suitable (Fig. 4, 5, 6).

3.3.6 Cleaning and Change of Tanks

The sediment containing dead bodies of both larvae and food organisms, metabolites and faeces of larvae, must be removed and cleaned from the bottom of larval rearing tanks regularly. Change of tanks, namely, transferring the larvae from the dirty tanks to the clean tanks has been observed to be a very effective and important technical measure for the larval rearing. The procedures and equipments are similar to those described above, except for the different mesh size of sieve.

From D-shaped larval stage to metamorphosis, two or three changes of tanks are enough to keep the water in good quality under the normal rearing density. If the density of larvae is higher than normal, change of tanks should be carried out every two to three days.

3.3.7 Water Replenishment

During the early period of rearing, water in the rearing tanks is replaced two times a day and about one-third or two-thirds of the volume is drained out and refilled each time. In the later period, especially when the spat collectors have been put into the tanks, the amount of renewed water should be increased based on water quality and growth rate of larvae.

3.3.8 Feeding

Isochrysis galbana, *Dicrateria* sp., *Phaeodactylum tricornutum*, *Platymonas* sp. and *Chlorella* sp. are the main food organisms for the larvae of bay scallop. Once replenishment of water is completed, the food organisms should be fed as soon as possible. Besides this initial feeding the larvae should be fed five more times over 24 hours.

The larvae may sink to the bottom of tanks in the case of overfeeding, while under-feeding may reduce the growth rate of larvae. Therefore, calculating the suitable concentration of algal cells is a key measure for the rearing of larvae.

The following formula is usually used to calculate the daily feeding rate for the larvae of bay scallop.

$$V_1 = \frac{C_2}{C_1} V_2$$

Where: V1: volume of water containing unicellular algae
V2: total volume of water in the rearing tank
C1: concentration of unicellular algae
C2: desired concentration of unicellular algae in the rearing tank

i. Daily observation of larval growth and development.

During the period of larval rearing, the feeding behavior and growth of larvae are observed every morning before the water is renewed. In general, the normal larvae are usually swimming in the upper and middle layers of the water. The feeding ration can be adjusted according to the food content in larvae's stomach. The number and shell length of larvae should be counted and measured every two or three days.

The growth and development of larvae are closely related to salinity, temperature, rearing density, water quality and food supply. Under suitable rearing conditions as mentioned above, the eye spot, which is located in the stomach region, will appear 10 days after fertilization, with the shell height at 180 to 190 μ m. In poorly fed, crowded or generally neglected culture, the beginning of spat stage is usually delayed and the time difference between beginning and end of settling is considerably extended. Sometimes, on account of culture under unsatisfactory conditions, the larvae are unable to metamorphose and eventually die.

3.4 Spat Collection

The appearance of eye spot is an indication that the larvae are approaching the settling stage. Once the eye spot appears, the larvae should be sieved and transferred into another well-cleaned tank. The spat collectors are then put into the tank for larvae to settle on as soon as the transfer is completed. Two kinds of collectors are commonly used in China. One is made of palm fibre rope and the other of pieces of polyethylene net.

3.4.1 Palm Fibre Rope Collectors

With a high tensile strength, good resistance to rotting, large surface area per unit, amenability to easy handling and lack of toxic substances, the collector made of palm fibre ropes is the ideal one for collecting spats of bay scallop. Before using, the palm rope should be thoroughly cleaned to remove harmful organic compounds, especially tannic acid. The procedure for processing and cleaning palm fibre ropes is as follows:

First, the newly twisted coir rope must be made pliable. This is done by "dry hammering", in which the rope is pounded with a special hammering machine. This process removes all remaining palm bark and other undesired fragments and makes the palm rope very flexible and thus easy to handle.

After soaking, the palm rope should be pounded again in a procedure called "wet hammering", which uses the same hammering machine as in dry hammering. During hammering, the rope should be sprayed with freshwater to wash away any exuded substances.

After wet hammering, the rope should be boiled in large vats for three to five hours and left soaking in the water overnight. Finally the rope is washed in clean freshwater and dried in the sun.

A collector is composed of a cluster of curtains. Each curtain is made of palm rope of 3 mm diameter and unit weight of 3 grams per metre. Each cubic metre of water needs a number of collectors equal to 1,500 metres in length of palm rope under normal conditions.

Although efficient in collecting spats, the palm rope collector is unsuitable in turbid sea regions, since it is easily silted by mud. The heavy silt on the collectors may cause heavy spat mortality during the intermediate culture period in natural sea region.

3.4.2 Polyethylene Net Collectors

Besides being easily processed, the collectors made of pieces of worn out polyethylene net can keep the water quality better than palm rope. Furthermore, it is especially suitable in turbid sea area regions, since it is not easily silted by mud.

The procedure for processing and cleaning the polyethylene net is as follow:

First, it is soaked in a solution of 0.5–1.0 % NaOH (sodium hydroxide) for at least one hour and then pounded and cleaned. After that, the pieces of net are washed in clean fresh water and dried in the sun.

About 2.5 kg worn out pieces of polyethylene per cubic metre (2.5 kg/m³) is considered to be suitable for spat collection.

Collectors must be distributed evenly in the tank. The tank bottom is usually covered by a layer of collectors. A number of horizontal and parallel polyethylene ropes are placed side by side over the tank. Collectors are vertically hung from the horizontal ropes and weighed down with small stones at their lower ends. This way, the collectors can move up and down freely with the fluctuations in water depth, especially during the period when water is renewed. Once all larvae get settled, food ration and illumination should be increased to the recommended levels.

Before the juvenile scallops are transferred from the rearing tanks to the sea for their intermediate (nursery) culture, the temperature of water should be decreased by 1–2 °C each day until it is close to that of the natural sea.

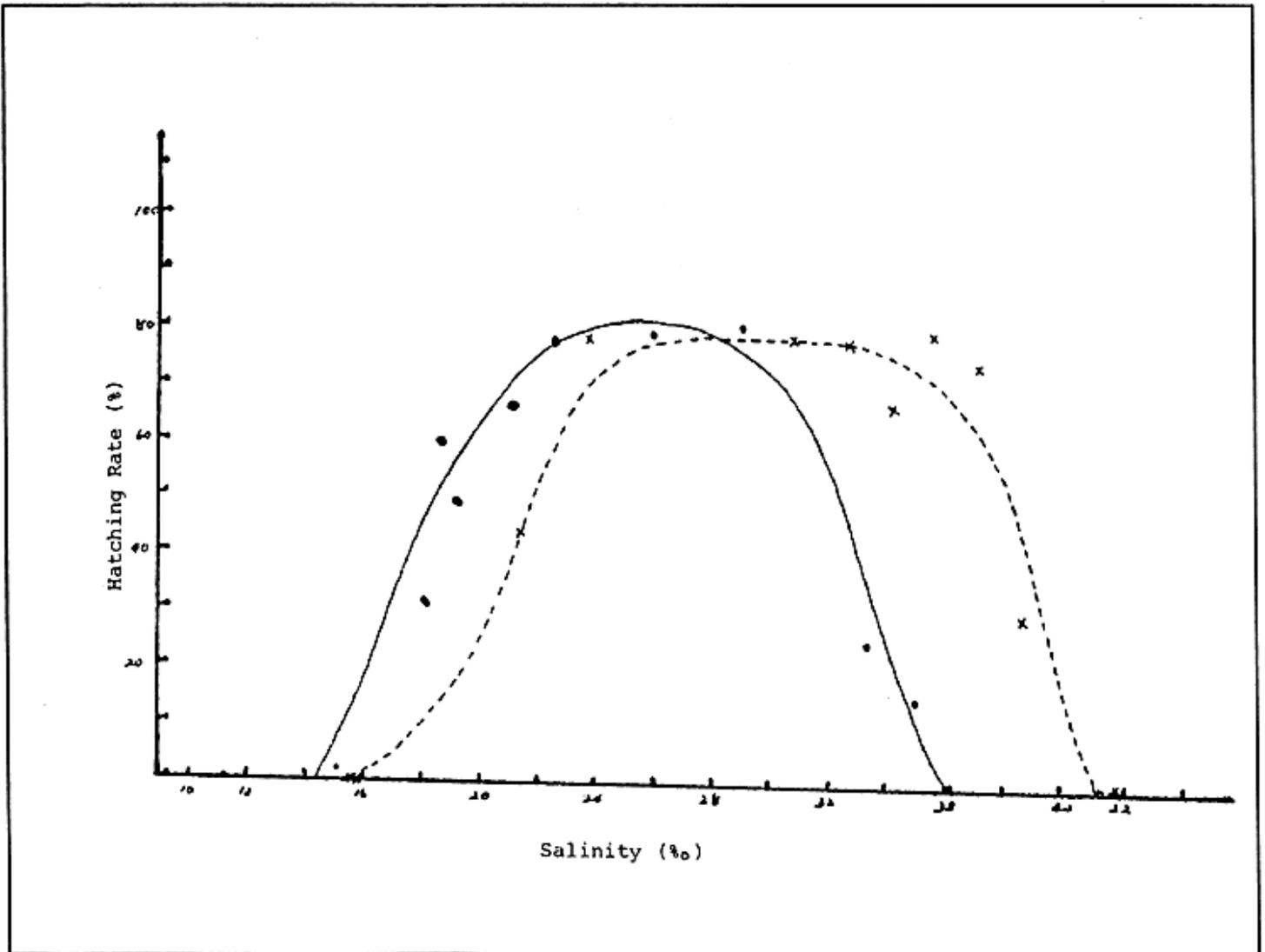


Figure 3. Effects of salinity on eggs of bay scallop *Argopecten irradians*.

- ——— • from fertilized eggs to D-shaped larvae.
- X ——— X from trochophore to D-shaped larvae.

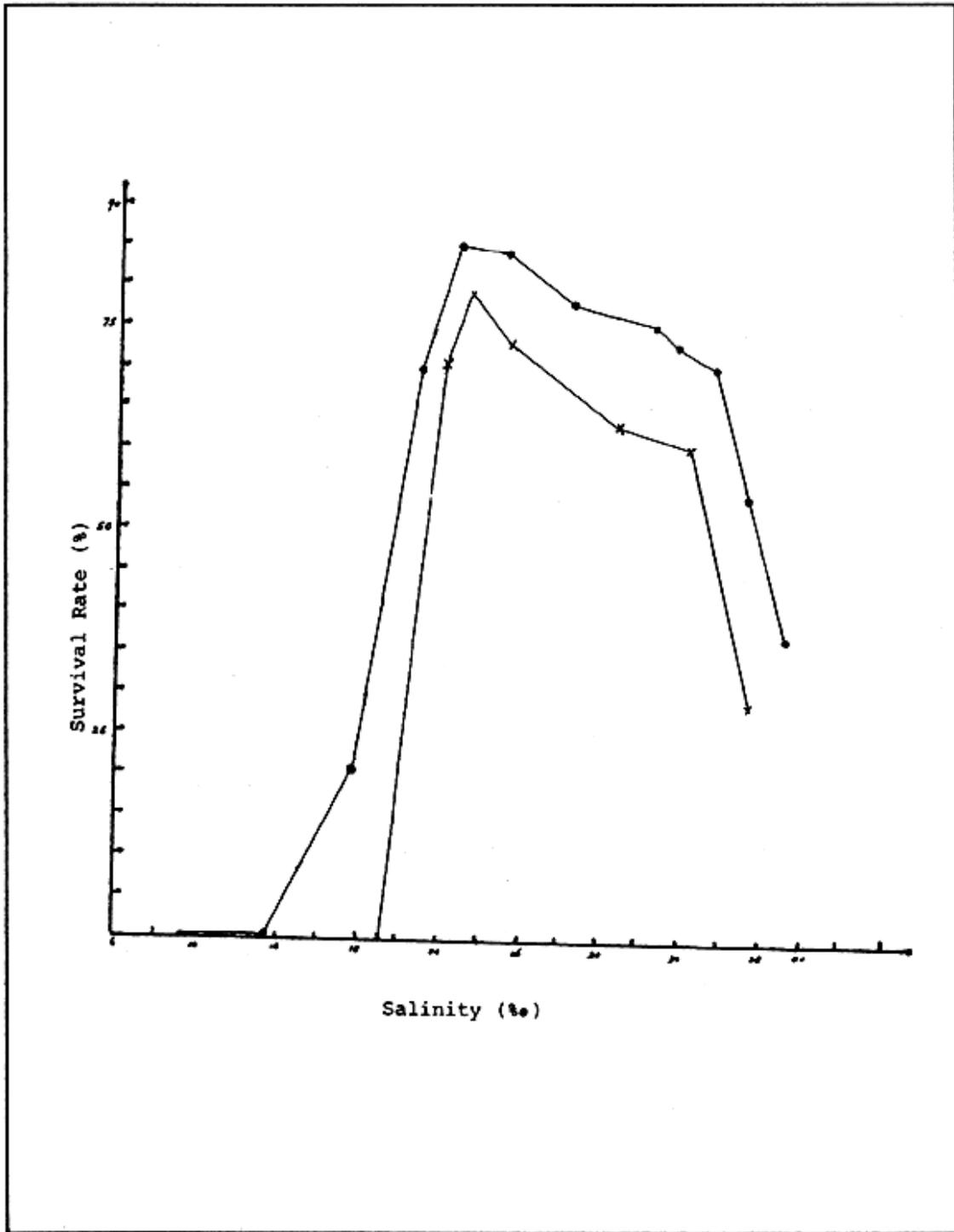


Figure 4. Effects of salinity on larvae of bay scallop *Argopecten irradians*.

- survival rate in 72 hours from fertilization.
- X——X survival rate in 144 hours from fertilization.

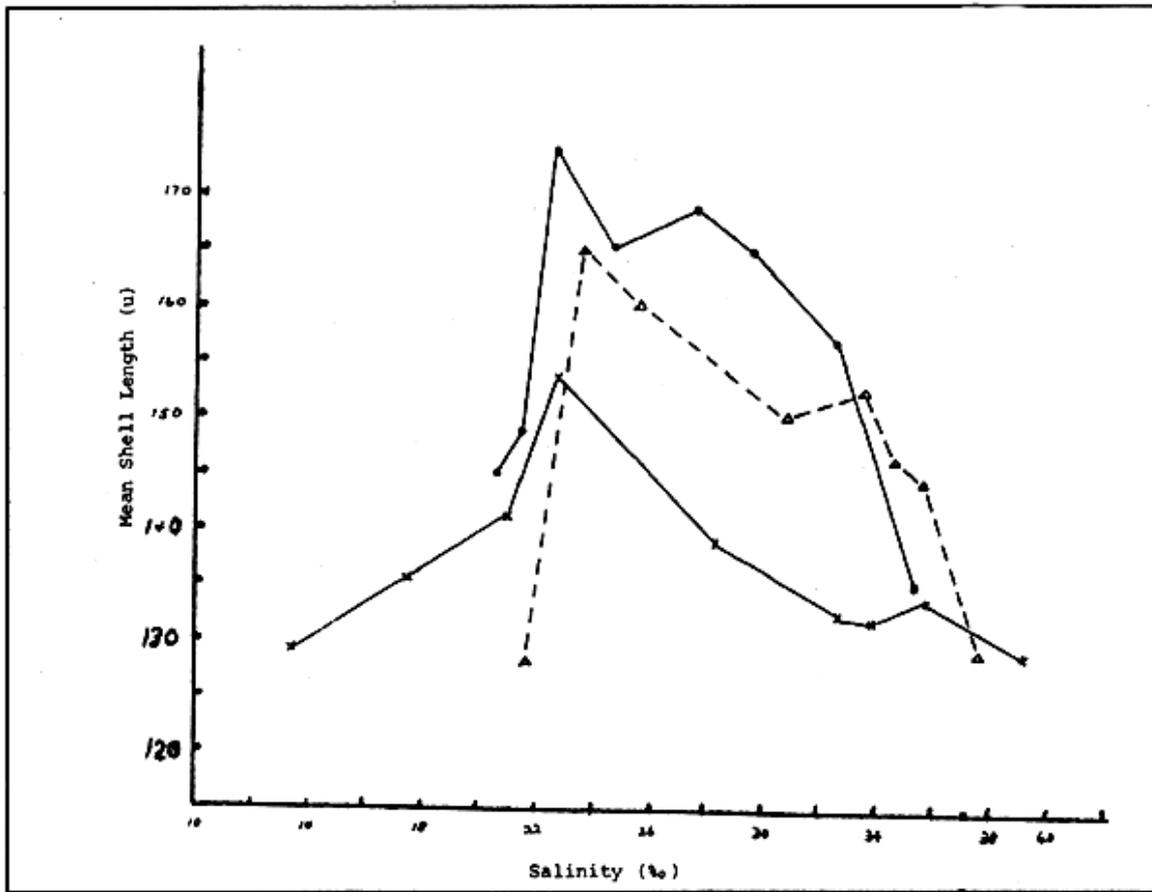


Figure 5. Effects of salinity on growth of larvae *Argopecten irriadians*.
 X—X mean shell length in 72 hours from initial size of 120•
 •—• mean shell length in 140 hours from initial size of 135•
 ▲—▲ mean shell length in 164 hours from initial size of 109•

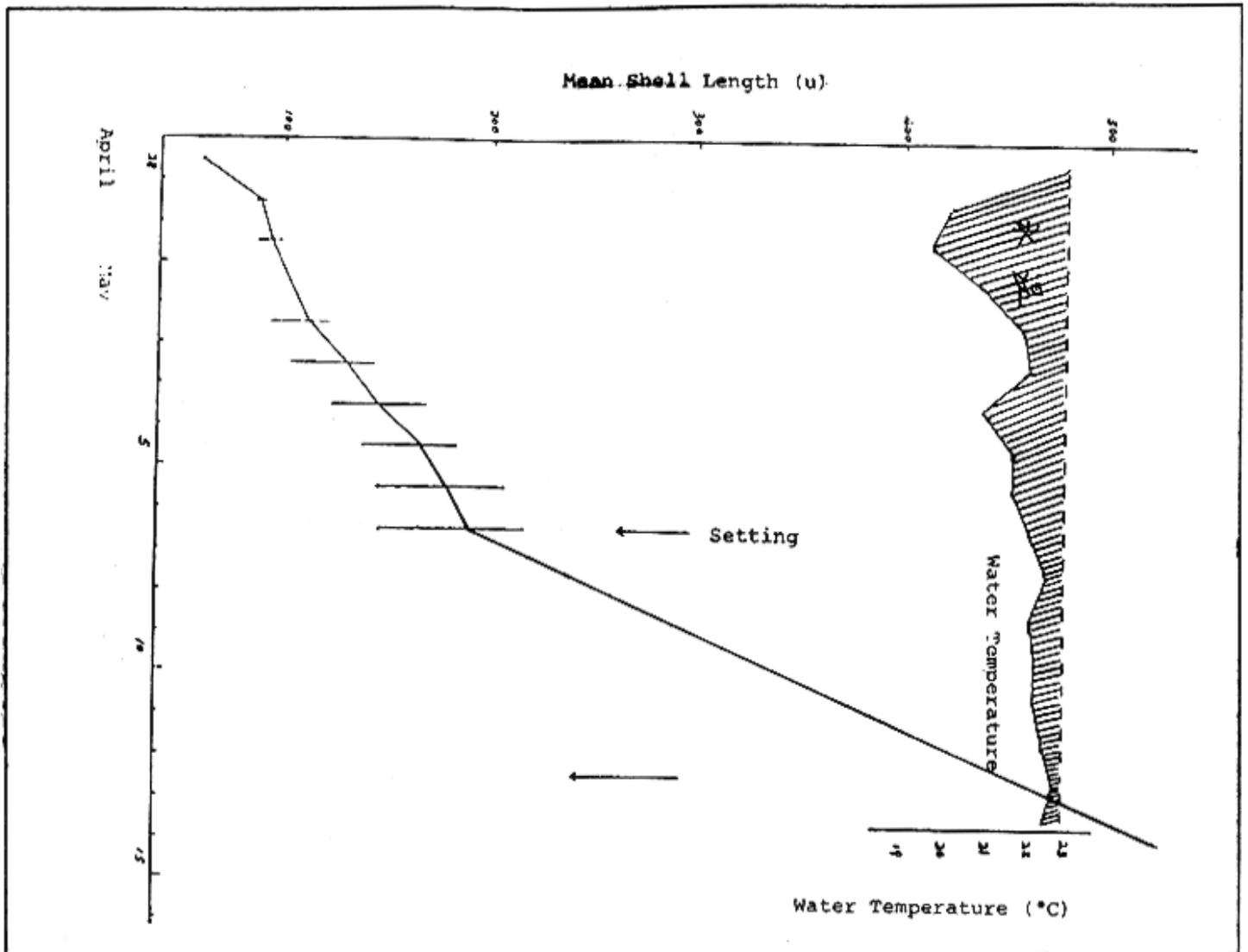


Figure 6. Growth of *Argopecten irradians* larvae throughout the whole rearing period (1985).

CHAPTER IV NURSERY CULTURE

4.1 The Importance of Nursery Culture

Nursery or intermediate culture involves transfer of the spats to the open sea and rearing them until they attain 5 mm in shell height.

Besides establishing more hatcheries, increasing production per unit area and development of multi-crops larval rearing, it is very important to increase the survival rate in nursery culture for producing enough spats to meet the demands of culture.

Because of the differences in the size of spats, condition of the sea area, culture materials and management, high mortality occurs after the spats are transferred to the open sea. At present, the survival rate in nursery culture ranges from as low as 10 % to 30–40 %.

4.2 Nursery Culture Technique

4.2.1 Spat Size for Nursery Culture

The size of the spat depends on the materials used, net mesh, water temperature, etc. In about 20 days after fertilization, when the shell height reaches 400–600 μm , the spats can be transferred to the sea.

When all the spats have settled, the rearing water temperature should be lowered by 1–2 °C every day to approximate the temperature of the sea. This is important for increasing their survival. Each transfer leads to better survival and better growth rate. The water temperature of the sea area must be higher than 10 °C when the spats are transferred.

4.2.2 Material for Nursery Culture

Two kinds of materials are used at present. One is a plastic pipe, 60 cm long and 25 cm in diameter, covered with plastic net (mesh smaller than the shell height of the spats) at both ends. The other is made of polythene bags. The size of the bag depends on the size of the spat collectors and is generally 30×40 cm. Every 8–10 bags are strung together on a rope. The bags should be separated to prevent them from rubbing each other in the sea.

4.2.3 Site Selection

The following factors should be considered in choosing a site for nursery culture.

- i. sheltered from strong wind
- ii. adequate tidal exchange
- iii. plenty of natural food organisms in the water
- iv. pollution-free
- v. water depth from 5–12 m, over a flat muddy-cum-sand bottom
- vi. transparency 1.5–2 m
- vii. stable in pH and salinity and without freshwater drainage
- viii. near the hatchery, to shorten the time of transfer

4.2.4 Culture Method and Density

Floating long-line rafts that are set transverse to the current and 60–70 m in length are used for nursery culture. The distance between the rafts is 8 m, while that between two culture lines is 0.5–1 m.

The culture density significantly influences survival. The spats grow fast and have high survival in low density. Generally, a suitable density per plastic pipe is 100,000 spats, while for a 30×40 cm polypropylene net bag of 40 or 50 mesh it is 10,000–30,000 spats, depending on the sea area condition.

4.2.5 Culture Management

The timing and the thinning out the growing spat also influences the growth and survival of the spats. The juvenile scallops should be distributed to more bags of certain mesh on time according to their growth.

The nursery culture in the sea may be divided into two phases. The first is from spat transfer to the first thinning when the shell height reaches 1–3 mm. The second is from the first thinning till the spats reach the marketable size of 5 mm.

First phase of nursery culture. There are two methods in this phase. One is to use 40×40×70 cm netcage. The other uses 30×50 cm polypropylene net bag. The former contains one palm screen spat collector and three spat-free collectors of the same material. The collectors are hung horizontally in the cage. The latter

contains half a spat collector and 15–20 g polypropylene net piece of 1–2 cm mesh to extend the bag. Survival in the first phase nursery culture is about 30 %.

Second phase nursery culture. The second phase nursery culture uses 30×50 cm net bag of 1 mm mesh, extended by net piece. About 2,500 spats are put into each bag and every ten bags are linked on a string. When the shell height reaches 5 mm, the spats may be sold.

The management practices directly affect the survival of spats during their culture in the sea. The bags should be cleaned every 5–7 days, depending on the turbidity of the water. The operation should be done carefully to prevent the scallops from falling off. The mud outside should be washed off gently. The cage should not be taken out of the water and the cleaning should be done from bottom to top. Cleaning time and frequency depend on the amount of silt. Cleaning improves water exchange, which enables the scallop to maintain a good filtration rate to obtain adequate food organisms.

4.2.6 Timing of Spat Transfer

Transfer of the spat should be done in the morning or evening. The spat bags should be laid over canvas or straw bags soaked with seawater and covered with a plastic or canvas sheet, a layer of straw bags soaked in seawater and a layer of canvas. Exposure of the spats to wind, sun and rain should be avoided.

The various transport links should be well coordinated to ensure a quick and smooth transportation to the culture ground.

CHAPTER V GROW-OUT CULTURE OF THE BAY SCALLOP

Grow-out culture, during which the juvenile scallops (shell height 5 mm) are reared to marketable size, takes about six months (June–November).

5.1 Biology

5.1.1 Growth and Life Span

The bay scallop has a fast rate of growth. The juvenile scallops produced in May can reach marketable size at the end of the year. If the reproduction time is advanced to March the shell height at the end of the year would be more than 6 cm, which is the more preferred marketable size. In a warmer sea area, the scallop grows faster with a growth rate of about 1 cm per month. It grows slowly when the temperature is lower than 10 °C and would stop growing below 5 °C. However, even if water temperature drops and growth slows down from October, the soft body weight of the scallop still increases. Around August, the high growth period, the shell grows at an average rate of 0.4–0.6 mm per day.

Epiphytes and other epicommsal animals are harmful to the growth of the scallop. Silt is deadly to juveniles with shell height of less than 1 cm. The scallop grows faster in slow current. Experiments have shown that the scallop grows fastest when the current velocity is 1–5 cm per second. Although scallop can secrete byssus throughout its lifetime, the older individuals secrete only rarely. The scallop can swim at all sizes. The bay scallop has a high filtration rate, with an average of 24.4 l/hr. Its average life span is about 12–16 months and a few can live up to 18 months, but rarely more than 24 months. A large number of scallops will die after their first reproduction. Their biological minimum size is 2.2 cm. If the harvest time has to be delayed to the next year, it must be made before April, or the yield and quality would be lower.

5.1.2 Condition Index and Adductor Rate

The visceral mass condition index is the ratio of the dry soft body weight to shell weight. The visceral mass condition index of the scallop cultured in Jiaozhou bay shows an upward trend from late April, followed by a downward trend at the middle of May and upward trend again in May. At the end of May, it reaches the maximum peak of the year, followed by a rapid drop in June. It goes up again at the middle of July and reaches the autumn peak in September. In late September, it drops gradually and becomes stable. The visceral mass condition index of the bay scallop is closely related to the development of the gonad and its reproductive cycle.

The percentage of fresh weight of the adductor to fresh soft body weight is defined as the adductor rate or adductor gain. Experiments have shown that the adductor rate of the bay scallop in the Jiaozhou Bay is more than 11 % in October and reaches above 13 % in November. It is lower than 8 % from June to August and drops to its minimum of about 5 % in June. The taste is also poor at this time.

As can be made out from the above account, the seasonal variation of the adductor rate is slightly ahead of the condition index. This is probably because the development of the gonad necessitates the transfer of nutrients from the adductor.

5.1.3 Mortality

The extent of mortality during grow-out culture would depend on the technical expertise of the handling personnel. The mortality during nursery culture and early grow-out culture, when the shell height increases from 5 mm to 1.5 cm, ranges from 20–30 %. After that the mortality rate comes down to 5–10 % towards the end of the year, if fouling organisms are removed regularly. However, inadequate management, typhoon and tremendous variations in environmental conditions as well as pollution could cause mortality of 30–50 %.

5.2 Ecology

5.2.1 Temperature

Laboratory experiments have shown that the bay scallops can tolerate temperature as low as -1 °C and as high as 31 °C. They will stop growing at a temperature lower than 5 °C and grow slowly at temperatures less than 10 °C. They will grow faster at the temperature range of 18–28 °C.

5.2.2 Salinity

The tolerable salinity range of the bay scallop is 16–43 ppt, with 21–33 ppt as the optimum range. It cannot adapt to sharp changes in salinity.

5.2.3 Culture Layer

The bay scallop grows well at the middle layer of the shallow sea; fairly well at the surface layer and poorly at the bottom layer. The preferred layer is 2–3 m below the sea surface.

5.2.4 Organic Carbon Content

The organic matter in seawater includes particulates, detritus and plankton. When the organic content is 150–398 •gc/1, the production of scallop can reach 45 to 52.5 t/ha.

5.2.5 Fouling Organisms and Predators

During the culture period, the cage may be attacked by many kinds of fouling organisms, such as

Tubularia marina, sponges, bryozoans, Polysiphonia, Enteromorpha, tunicates (ascidians), mussels, oysters and barnacles. These organisms plug the cage and block the water exchange. The epicomensal animals also compete for food and even cause diseases. Culture experiments have shown that fouling by oysters and barnacles is much less in areas far from the coast. The quantity of foulers attaching to the cage in water layer deeper than 3 m is only 10–20 % of that in the surface. At the sea bottom, fouling is practically nonexistent.

The predators include starfish, sea urchin, crab, octopus, etc., which must be removed immediately when discovered.

5.3 Grow-out Culture Technique

5.3.1 Culture Site Selection

The areas used for Laminaria, Mytilus and Chlamys farreri culture are suitable for bay scallop culture. The general requirements are given below:

- i. The area should have fertile water and adequate tidal exchange.
- ii. The water depth should be more than 10 m, with flat bottom of mud-cum-sand.
- iii. It should be pollution-free.

5.3.2 Culture Cages

The lantern cage has been found to be the most economical, durable and easy to handle. The cage is a net tube woven with 6–12 ply polyethylene thread, separated into 7–8 chambers by plastic discs of 30 cm diameter with some round holes on them. There is a space of 15 cm between every two discs. The number of chambers depends on the depth of the water. The cage is generally about 1.4–1.5 m in height.

5.3.3 Culture Management

An important step in grow-out culture is the need to prevent overcrowding of the growing juveniles to ensure proper growth. When the juveniles have grown to 1.5 cm in the nursery bags, they are taken out and distributed on to a number of grow-out cages. About 25–30 juveniles are usually stocked in each chamber of the cage. This works out to an average stocking density of 1.5 million juveniles per hectare. The cage has two layers of netting, the inner of 2–3 cm mesh and the outer of 1–3 cm mesh. When the scallops grow to 2–5 cm in shell length, the outer layer is removed. This method serves to reduce labour and overall production cost, which otherwise would be more if the scallops are to be transferred to new cages. The removal of the outer layer also helps in getting rid of the fouling organisms.

The juvenile scallops are generally placed in grow-out cages around July and are harvested in November or December, after about 5–6 months. During this period, fouling organisms should be removed. After September, with the growth of the scallops, the flotation capability of the main raft lines must be increased. The hang links should be checked regularly to prevent the cages from touching the sea bottom and getting damaged by wear and tear.





PART II

SEA CUCUMBER (STICHOPUS JAPONICUS) CULTURE IN CHINA

CHAPTER I BIOLOGY OF THE SEA CUCUMBER

Sea cucumbers, which have been collected as food organisms for a long time, belong to class Holothuroidea of phylum Echinodermata. There are over 1,100 species of sea cucumbers under six orders.

1.1 Taxonomy of Holothuroidea

| | |
|---------------|--|
| Aspidochirota | Number of tentacles 15–30, but usually 20. Tentacle vesicle, tube feet with terminal suckers and respiratory trees present. Most of the edible sea cucumbers, such as <u>Holothuroidea nobilis</u> and <u>Stichopus japonicus</u> belong to this order. |
| Elasipoda | Flat ventral side. Usually it has a groove between dorsal and ventral sides, occasionally has tail; 10–20 tentacles without vesicles. Degenerated tube feet without suckers in 1–2 rows. Respiratory trees absent. Species in this order mostly distributed in deep sea, e.g. <u>Psychropotes longgicauda</u> , which lives at depths below 1,100 m in the South China Sea. |
| Pelagothurida | Floating mode of living. Tentacle vesicle present. Tube feet absent. e.g. <u>Pelagothrid</u> . |
| Dentrochirota | Branching tentacles, without tentacle vesicle. Tube feet and respiratory trees present. e.g. <u>Cucumaria echinata</u> . It is melon-like in shape, 3–4 cm in length, with 10 tentacles. Tube feet along five radial canals in two regular rows. It inhabits seashore or intertidal zones. It is known as “Sea peanut” in the Eastern Guangdong Province, and usually is fed to chicken. |
| Molpadonia | Smooth body surface, posterior part of body thin and tail-like. Anus at the end of the tail. Tube feet absent. 15 tentacles, not branching or divided into two. Tentacle vesicle and respiratory trees present. Example: - <u>Acaudina molpadioides</u> (Semper): Large body with smooth surface. Tube feet absent. Tentacle 15 in number and not branched. Distribution in sandy bottoms in about 20–50 m depth in the East China Sea. After drying, the processed, known as “Xiang Shen” is sold. - <u>Paracaudina chilensis ransonneti</u> (V. Marenzeller): Popular name: “Sea rat”. Spindle shaped body with long posterior tail. Whole body smooth without tube feet. Tentacles are 15, each of them with four finger-like branches. Living in subtidal sand caves. From Dalian, Liaoning Province to Zhanjiang, Guangdong Province, it is widely distributed in China and especially abundant along the Yellow Sea. |
| Apoda | Wormlike forms. Finger-like or pinnate branching tentacle, 10–20 in number. Tube feet and respiratory trees absent. e.g. <u>Patinaptaooplax</u> (V. Marenzeller). It has 12 tentacles, each having 4–5 pinnate branches. Commonly distributed in the intertidal zone in North China. |

There are more than 100 species of Holothuroidea in China. They are distributed mostly in the South China Sea and Xisha Archipelago. There are about 20 edible species and the most preferred is Stichopus japonicus. The following 5 species are also commercially valuable sea cucumbers.

- Thelenota ananas (Jaeger): Large body, reaching a maximum length of 1 m. Its dorsal caruncles connected to each other like petal, so that it is known as “Plum blossom cucumber”, or “Pineapple cucumber”. Alive, its body colour is orange-yellow or orange-red on its dorsal side and red on its ventral side. Inhabits coral reef and co-exists with pearl fish in its cloaca. Only distributed in Xisha, Zhongsha and Dongsha Archipelago of China.
- Stichopus variegatus: Square shaped body, dorsal caruncles lining irregular. Little variation in body colour, usually dark yellow with olive dots. Found in intertidal coral reefs. The maximum length can be 40–50 cm, usually found in deeper waters. Distribution: Hainan Island, Leizhou Peninsula and along the coast of Xisha Archipelago.
- Holothuria scabra (Jaeger): Rough body surface, few tube feet. Dorsal side is dark brown-green, ventral side is white. Usually living along coastal reefs or in sandy areas where seaweeds are lush and tidal current is strong. Can reach a maximum length of 70 cm. Distribution: Hainan Island and Dongxing off Guangxi Province.
- Holothuria nobilis (Selenka): Also known as “Wuyun Shen”. Usually there are several breast-like caruncles on its body. Found under the sand with seaweeds in fort reef. Sometimes on seaweed in coral reef. Only distributed in Xisha Archipelago and Xinchun of Hainan Island.
- Actinopyga mauritiana (Quoy et Gaimard): 25–27 tentacles, lined irregularly. Around the anus there are five small calcareous teeth. Dorsum is olive-brown when it is alive. Each tube foot has a white ring. Distribution: Xisha Archipelago and Hainan Island.

1.2 Nutritional and Medicinal Values of S. japonicus

1.2.1 Nutritional Value

China is one of the earliest countries where sea cucumbers were eaten. Sea cucumbers are known their high protein content and absence of cholesterol. They are also considered as a tonic food. According to laboratory analysis, soaked Stichopus japonicus contains 76 % water, 21.5 % protein, 0.3 % fat, 1 % carbohydrate, 1.1 % ash, and 118 mg of calcium, 22 mg of phosphorus and 1.4 mg of iron per 100 g body weight. Dried S. japonicus contains 6 mg of iodine per kg body weight. Its intestine contains 72.49 % water, 8.836 % crude protein, 2.687 % crude fat and 15.987 % ash.

1.2.2 Medicinal Value

In China, sea cucumbers have been used as a beneficial drug since the Ming Dynasty. The body wall of S. japonicus is known to cure kidney

diseases, constipation, lung tuberculosis, anaemia, diabetes, etc. Its viscera is said to be a cure for epilepsy and the intestine has some curative effects on stomach and duodenal ulcers.

According to current medical research, the dermal connective tissues, body cavity membrane and corium inner gland tube of *S. japonicus* contain many kinds of acidic mucopolysaccharide, that can have special effect on growth, recovery from illness, anti-inflammation, bone formation and prevention of tissue ageing and arteriosclerosis. Mucopolysaccharide is also an extensive anti-tumour drug. Through abdominal or intravenous injection, it was seen to have apparently suppressed the transplanted experimental tumours, S-180, S-37, etc. At the same time, it also has intensive effect on contravariant. Holotoxin extracted and purified from sea cucumber is an effective antimycin. It can suppress many kinds of molds at the density of 6.25–25 •g/ml.

So far, the species that have been known to have similar medicinal effects as *S. japonicus* are *Stichopus variegatus* Semper, *Stichopus chloronotus* Bramde, *Thelenota ananas* Jaeger and *Bohadschia argus* Jaeger.

1.3 Morphology

1.3.1 External Characteristics

Tube-shaped body, 20–40 cm in length and 3–6 cm in width. Quadrilateral in transverse section. Flat ventral side, on which tube feet are lined in three irregular longitudinal rows. Dorsum slightly raised with irregular caruncles in 4–6 rows. Mouth anterior and inclined to dorsum. There is a convex area behind the mouth, known as the gonophore.

1.3.2 Internal Structure (Fig. 1)

a. Body Wall

The cuticle is the external protective layer, under which there is the dermal cortex in which the ossicles are embedded. The ossicles within the body wall are table-like in shapes. The bases of table-like ossicles are either low or not completely developed and only have perforated trays. The muscular layer consists of transverse and longitudinal muscles. Under the muscular layer, there is a thin membrane, known as body cavity or mesentery, which is connected with the intestine. This membrane is divided into three segments: dorsal mesentery, left mesentery and right mesentery.

b. Digestive System

The digestive tract is a longitudinal tube and winds two times in the body cavity. It includes the following organs: tentacles, mouth, pharynx, oesophagus, stomach, intestine, general cloacal cavity and anus. The mouth is anterior and lacks masticatory organs. Twenty tentacles, which help in feeding, surround the mouth of *S. japonicus*. There is a calcarious ring around the pharynx, which is followed by a short oesophagus and an elastic stomach. The part where the oesophagus joins the stomach contains a lot of red pigments. The digestive tract gets easily broken at this point. Next to the stomach is the first small intestine (descending intestine), which abounds with yellow pigment and accompanies a dorsal blood vessel network. The end of the first small intestine extends forward to the left and appears “U”- shaped. This part is called the second small intestine (ascending intestine). The dorsal blood vessel network attaches to the second small intestine and closely connects with the left respiratory tree. The end of the second small intestine connects with the large intestine. The large intestine lies along the centre of the longitudinal muscle and directly reaches the general cloacal cavity, around which are muscles that enable its expansion and contraction. The external opening of the general cloacal cavity is the anus.

c. Respiratory System

With a thin lateral wall, the general cloacal cavity is a wide and short tube which stretches outwards, divides into two branches, and extends into the body cavity. The branches are called respiratory trees because they look like trees. The “trees” absorb oxygen. The dorsal blood vessel network is distributed to the outside of the left respiratory tree. The absorbed oxygen enters into circulatory system through the respiratory trees and is carried into other organs by the blood. Carbon dioxide (CO₂) is removed out of the body along the same channel.

Apart from the respiratory trees, the skin also has a respiratory function. Because the walls of the tube feet in ventral side are very thin, the feet could absorb oxygen from the water and remove CO₂ from the body.

d. Circulatory System

There is a hemal ring around the esophagus, from which five radial blood vessels are divided and spread along five canals and under muscle layer, until the posterior end. The dorsal blood vessel and the ventral intestinal blood vessel form the blood network, which covers the loops of the intestine. The left respiratory tree closely connects with the blood network. The blood of *S. japonicus* is transparent and brown in colour.

e. Water-vascular System

The water-vascular system is located above the haemal ring and surrounds the oesophagus. Five radial canals branch off from the ring canal and send branches forward to tentacles and backward to the tube feet along five ambulacral zones. The ring canal also connects with the Polian vesicle and the stone canal. There is a small pore at the end of the stone canal.

f. Nervous System

Oral nerves: There is a nerve ring in the calcareous ring from, which five radial nerves extend. They send branches forward to the tentacles and backward to the tube feet along the ambulacral zone.

Inner nerves: Nerve ring is absent. There are only five radial nerves, which give off branches to the transverse, longitudinal and radial muscles.

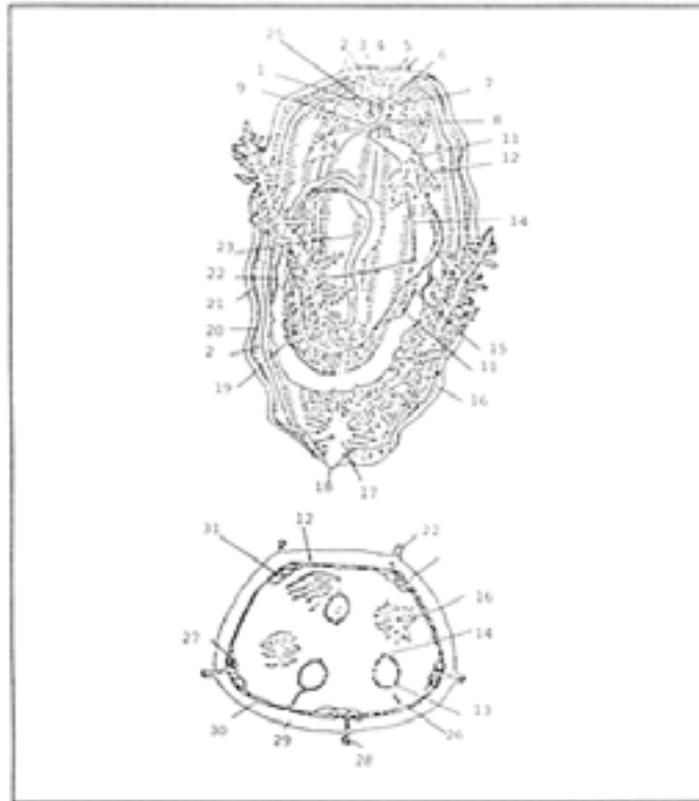


Figure 1. Internal structure of a sea cucumber. (1. pharynx wall; 2. radial water vessel; 3. tentacle vessel; 4. tentacle around mouth; 5. calcareous ring; 6. inner maduciorite; 7. stone canal; 8. water-vascular ring; 9. haemal ring; 10. oesophagus; 11. dorsal blood vessel; 12. gonad; 13. descendant intestine; 14. ventral blood vessel; 15. blood vessel joint; 16. respiratory tree; 17. general cloacal cavity; 18. anus; 19. blood vessel network; 20. longitudinal muscle; 21. vesicle; 22. ascendant intestine; 23. rectum; 24. Polian vesicle; 25. mouth; 26. mesentery; 27. water-vascular canal; 28. tube feet; 29. body wall; 30. radial muscle; 31. radial nerves).

1.4 Ecology

1.4.1 Distribution

S. japonicus is widely distributed. Its vertical distribution is from intertidal zone to 20–30 m depth zone. It is mainly distributed in the West Pacific Ocean. The Northern limits of its geographic distribution are the coasts of Sakhalin Island, U.S.S.R. and Alaska, U.S.A. The Southern limit is Tanega-shima in Japan. In China, it is commonly distributed on the coast of Liaoning, Hebei and Shandong Province, Yantai and Qingdao of Shandong Province. Its Southern limit in China is Dalian Island in Lian Yungang, Jiangsu Province.

1.4.2 Habitat

The environmental factors which have a close bearing on the habitat of *S. japonicus* are water temperature, salinity, tidal current, substratum, food, attachment and living spot of juveniles. Among them, water temperature, salinity and substratum are the main limiting factors.

a. Water Temperature

S. japonicus lives in the temperate and frigid zones. It cannot adapt to higher or lower water temperature. When the seawater temperature is below 3 °C, it moves slowly, feeds less and lives in semi-dormancy. Its feeding rate is also greatly reduced as water temperature rises to 17–19 °C, and the animal goes estivating as water temperature goes over 20 °C. If water temperature in its living environment remains high or low for a long period, it cannot grow normally.

b. Substratum

S. japonicus commonly lives on reefs and rocks. It is also found on muddy or sandy bottom with eel-grass clumps. However, it is hardly found in pure sandy or muddy bottom.

The distribution of *S. japonicus* depends on the particle composition of the substratum. Tables 1 and 2 show the distribution density of *S. japonicus* and the particle composition of the substratum in the coast of Ishigawa County in Japan and Gangdong in Qingdao, China, respectively. Investigations of the above regions show similar features. If the substratum contains more rough sand and rock the larger the distributive density of *S. japonicus*; less if the stratum is made up of small sand and mud.

Table 1. Investigating results of the coast along the Ishigawa County of Japan

| Investigated place | Water depth (cm) | Sali. Chlори. (‰) | Water temp. (°C) | Sorts and compositions of partacles(%) | | | | | | density of distribution (No./m ²) |
|--------------------|------------------|-------------------|------------------|--|-------|-------|-------|-------|-------|---|
| | | | | | 3.00 | 1.00 | 0.50 | 0.20 | | |
| | | | | > 3.00 | | | | | Mud | |
| 1 | 205 | 17.14 | 16.2 | 45.24 | 13.80 | 5.40 | 8.10 | 26.25 | 1.20 | 0.03 |
| 2 | 370 | 17.36 | 15.7 | 85.93 | 1.70 | 1.96 | 2.32 | 7.90 | 1.00 | 0.047 |
| 3 | 460 | 17.39 | 15.6 | 60.86 | 7.72 | 6.04 | 9.24 | 13.78 | 2.36 | 0.027 |
| 4 | 615 | 17.50 | 15.2 | 50.80 | 13.57 | 9.86 | 14.15 | 9.49 | 2.13 | 0.01 |
| 5 | 1650 | 17.11 | 15.3 | 0.00 | 0.00 | 1.26 | 24.78 | 55.42 | 18.54 | 0.003 |
| 6 | 1790 | 18.29 | 15.2 | 0.00 | 0.00 | 0.00 | 0.00 | 49.50 | 50.10 | - |
| 7 | 1840 | 18.32 | 15.1 | 0.00 | 0.00 | 0.00 | 0.00 | 36.65 | 65.35 | |
| 8 | 175 | 16.44 | 17.2 | 83.10 | 8.37 | 3.36 | 1.54 | 3.19 | 0.45 | 0.04 |
| 9 | 315 | 16.77 | 16.1 | 60.15 | 6.97 | 6.64 | 12.44 | 12.70 | 1.10 | 0.04 |
| 10 | 420 | 17.26 | 15.9 | 61.10 | 6.53 | 7.17 | 16.77 | 6.86 | 1.57 | 0.04 |
| 11 | 563 | 17.42 | 15.5 | 59.47 | 12.34 | 7.54 | 11.69 | 6.38 | 2.57 | 0.03 |
| 12 | 885 | 17.20 | 15.4 | 69.37 | 17.46 | 9.56 | 1.19 | 1.18 | 1.29 | 0.03 |
| 13 | 1765 | 18.03 | 15.2 | 0.00 | 0.00 | 0.00 | 0.00 | 46.93 | 53.07 | 0.003 |
| 14 | 1705 | 18.10 | 15.2 | 0.00 | 0.00 | 0.00 | 0.00 | 41.49 | 58.51 | - |
| 15 | 315 | 16.95 | 16.5 | 74.22 | 1.68 | 1.34 | 5.17 | 15.76 | 1.84 | 0.10 |
| 16 | 460 | 17.35 | 16.0 | 45.42 | 12.27 | 7.53 | 10.19 | 23.01 | 1.58 | 0.05 |
| 17 | 500 | 17.46 | 15.7 | 61.48 | 9.96 | 7.16 | 6.39 | 10.47 | 4.54 | 0.05 |
| 18 | 625 | 17.47 | 15.4 | 73.52 | 13.92 | 1.29 | 1.70 | 2.31 | 7.26 | 0.04 |
| 19 | 1385 | 18.04 | 15.2 | 0.00 | 1.54 | 3.21 | 30.19 | 55.10 | 9.96 | 0.02 |
| 20 | 1565 | 18.06 | 15.2 | 0.00 | 0.00 | 0.00 | 0.00 | 45.45 | 54.55 | 0.07 |
| 21 | 1580 | 18.07 | 15.2 | 0.00 | 0.00 | 0.00 | 0.00 | 46.01 | 53.99 | 0.07 |
| 22 | 465 | 17.53 | 15.4 | 30.73 | 21.09 | 14.97 | 8.82 | 22.42 | 1.97 | - |
| 23 | 590 | 17.51 | 15.4 | 49.51 | 13.77 | 8.46 | 6.74 | 19.62 | 1.90 | 0.08 |
| 24 | 1105 | 17.90 | 15.1 | 0.00 | 1.40 | 1.56 | 27.63 | 65.74 | 3.68 | 0.05 |
| 25 | 1135 | 17.95 | 15.2 | 0.00 | 0.38 | 0.40 | 46.56 | 48.13 | 4.52 | 0.06 |
| 26 | 1270 | 17.98 | 15.3 | 0.00 | 0.00 | 0.53 | 22.84 | 67.22 | 9.41 | 0.04 |
| 27 | 1490 | 17.98 | 15.2 | 0.00 | 0.00 | 0.00 | 8.81 | 66.05 | 25.14 | 0.02 |
| 28 | 1590 | 17.94 | 15.2 | 0.00 | 0.00 | 0.00 | 5.11 | 46.58 | 48.31 | 0.02 |

Table 2. Investigating results of Gangdong in Qingdao, China

| Releasing place | Water depth (cm) | Specific gravity (‰) | Water temp. (°C) | Sorts and compositions of partacles(%) | | | | | | density of distribution (No./m ²) |
|-----------------|------------------|----------------------|------------------|--|-------|-------|-------|--------|-------|---|
| | | | | | 0.9 | 0.45 | 0.3 | | | |
| | | | | > 0.9 | | | | < 0.25 | Mud | |
| I | 4 | 1.025 | 4.4 | 6.39 | 4.70 | 10.43 | 35.53 | 35.77 | 7.17 | 0.53 |
| II | 4 | 1.025 | 5.2 | 1.21 | 13.45 | 26.98 | 32.07 | 15.40 | 10.89 | 0.53 |
| III | 3 | 1.025 | 5.3 | 15.41 | 31.42 | 27.81 | 14.56 | 8.01 | 2.79 | 0.90 |
| IV | 5 | 1.025 | 4.9 | 1.65 | 4.26 | 13.32 | 37.32 | 37.26 | 5.95 | 0.58 |

* Results in March 6th, 1983.

c. Salinity

Generally, *S. japonicus* lives in seawater of normal salinity. Its suitable chlorinity range is 13.4–19.2 ppt. It can also adapt to certain habitats where it has lived for a long period. Living around reef and rock, the body colour of *S. japonicus* is red-brown or brown (hence called red sea cucumber). It adapts slightly to higher salinity and is mostly distributed in areas mainly influenced by oceanic water. Living on sandy and muddy bottom with bush seaweeds, its body colour is greenish yellow or greenish brown (thus also called green sea cucumber). It adapts slightly to lower salinity and is distributed in areas mainly influenced by fresh water.

Its tolerance of low salinity relates to its body colour. When chlorinity of seawater is below 5.1 ppt, both the red and green sea cucumbers show the tendency to die. The half-lethal time is 8–17 hours and the lethal time is 9–24 hours. In seawater with chlorinity level of 5.8–9.9 ppt, some of the green and red cucumbers die in a short period. The higher the temperature, the lower the intolerance, especially the red sea cucumber. At chlorinity level of 11.4 ppt, the green sea cucumber can be adapted for about 10–12 days at normally crawling stage. Over this period it can feed and grow normally. In the case of red cucumber, its tube feet initially lose their ability of adherence after 8–10 days and

some dead sea cucumbers with decayed dorsum can be found. Half-lethal time (LT50) is 14 days and all die after 22 days. When chlorinity is 12.6 ppt, the green sea cucumber lives normally, but at 50 ppt the red sea cucumber dies after 30 days. It can only live normally in seawater of 14.1 ppt.

d. Depth

S. japonicus is distributed from the intertidal zone to a depth of 20 m. At different depths, its body weight varies. Juveniles above 3–4 cm in length mostly stay on rocks in the intertidal zone or attach on large subtidal seaweeds. *S. japonicus* less than 50 g are distributed in shallow water along the coast: those of 50–100 g are found in 5 m depth; 150–200 g in 10–15 m; and above 200 g in the region more than 15 m deep. It is rare to find *S. japonicus* over 200 g living in intertidal zone or shallow water. On the other hand, juveniles are rarely found in water deeper than 10 m.

1.4.3 Feeding Habit and Growth

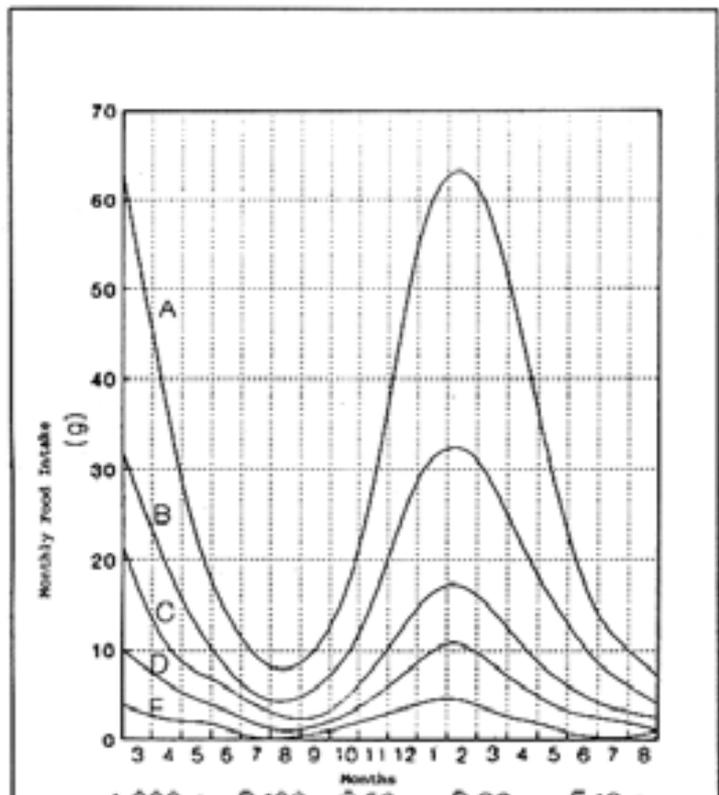
The food of *S. japonicus* includes unicellular algae (mainly benthic diatoms), protozoans, fish eggs, larvae of sea animals, organic detritus and other small organisms. There is also a lot of sand and pieces of molluscan shells in its digestive tract. The composition of ingested sand corresponds to that of its habitat substratum. It feeds without selectivity; whatever is clinging on its tentacles is brought to its mouth. *S. japonicus* has been found to feed on 1.5 × 0.7 cm size shell. The feeding habits of the juvenile and young of *S. japonicus* are closely related to their ecological conditions. Generally, the juveniles and young live under rocks or attached to seaweed in intertidal zone. Apart from some mud and sand, the digestive tract, has mainly benthic algae and detritus. Up to a body wall weight of 2.0–2.5 g, it remains attached. It changes over from attached to benthic conditions when the weight exceeds 2.5 g.

The feeding activity of *S. japonicus* is characterized by crawling with its tentacles, which at the same time convey food to its mouth. Generally, it feeds on materials found at the surface of the sea bottom, but it also dig deeper when hungry. Even the young (2–3 cm in length) can dig 3–4 mm into the mud or sand to feed.

S. japonicus feeds without intervals, but the amount of food intake during day and night are different. At daytime, the intake is low because of its inactivity, the intake is high during night. The day-night feeding ratio is about 3–4:7-6.

The food intake by *S. japonicus* and its tract length and weight vary according to seawater temperature. It goes with aestivation at water temperatures over 20 °C. At this stage, it stays under rock or rock crevices and stops moving and feeding. There is no food in its digestive tract, which is reduced to the shortest and lightest stage. After aestivation, it crawls from its shelter and feeds again at 19–20 °C. Its digestive tract recovers gradually, but the length and weight of the tract do not increase. When water temperature is below 3 °C in winter, its normal activity also slows down; its feed intake decreases. When water temperature rises above 3 °C in the following spring, it begins to move frequently and its feed intake increases. At water temperature of 8–10 °C, it moves at the maximum frequency. Its digestive tract can reach as much as 5.7– 6.4 times longer than its body length. At temperature of 17–19 °C, it goes into its reproductive phase. During this period, it feeds less, moves more slowly and its digestive tract degenerates.

Monthly variations in food intake in relation to body wall weight of *S. japonicus* are delineated in Figure 2. It can be calculated from this figure that the annual food intakes by individuals of *S. japonicus* with body wall weights of 10 g, 30 g, 50 g and 100 g are 0.8 kg, 2.1 kg, 3.5 kg and 6.8 kg respectively.



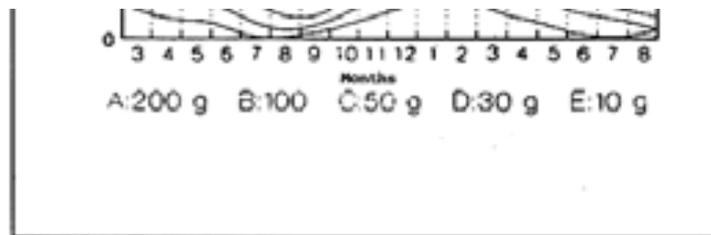


Figure 2. Monthly variations in food intake in relation to different body wall weights of *Stichopus japonicus*.

The retention time of food in the digestive tract of sea cucumber is 21 hours, during which only 52–54 % of organic carbon in the food is absorbed. Carbon digestibility in sea cucumber is poor when compared to that in fishes. The enzymes present in the digestive tract of *S. japonicus* are amylase, cellulase, pectase, proteinase, dipeptidase, fatty glycerol and high fatty acid.

The movements, feeding and growth of *S. japonicus* are limited by water temperature and the seasons. Its normal activities and feeding are confined to about half the year only. Hence, it grows slowly. The body length of an *S. japonicus* that hatches in June is about 5.9 cm after 12 months, while its body weight reaches 15.5 g. After two, three and four years, its body length and weight are 13.3 cm and 122.4 g, 17.6 cm and 307.1 g and 20.8 cm and 472.5 g respectively. It has been reported that *S. japonicus* could live at least five years.

1.4.4 Respiration

The respiratory trees in the body of *S. japonicus* are the main respiratory organs, the skin also possessing respiratory functions. Its oxygen consumption varies with temperature level. At its normal temperature range, the adult consumes about 0.4–0.8 ml of oxygen per hour.

For respiration the sea cucumber draws in water several times before expelling the respired water. At water temperatures of 11–14 °C, it draws in water 9–10 times before expelling the water once. At 19–22 °C, it expels water once after drawing in 9–15 times. At 8 °C, it only opens and closes its anus slightly. If the respiratory trees are cut-off, the sea cucumber cannot perform its respiratory activities at the anus. The extent of respiration performed by the skin depends on the temperature. It increases with increasing temperature and can constitute as much as 60–90% of total respiration.

1.4.5 Movement

S. japonicus crawls slowly on the sea bottom through constriction of its longitudinal and transverse muscles and tube feet, it can cover 1 m in 10 minutes. The maximum linear distance that can be covered during one day is 170–180 m (average: 140 m). With abundant food and good environmental conditions, it only moves about 5 m per day. However, if food is scarce and the environmental conditions are poor, it can move for quite a distance and even loosen its body and float with the waves. Under artificial cultivation also, both the juveniles or adults can float. Mainly, this occurs during the night or at dawn.

1.4.6 Predators

The natural predators of *S. japonicus* are few. Sea gulls prey upon it at the intertidal zone. *S. japonicus* has been found in the stomach of salmon and trout. Young sea cucumbers (about 3 g) have also been found in the stomach of some other fishes such as bullheads. Starfish may prey on juveniles less than 3 cm long.

1.5 Ecological Characteristics

1.5.1 Eversion of Viscera and Regeneration

Under poor environmental conditions, *S. japonicus* can evert its viscera, which includes stomach, intestine, respiratory trees, dorsal blood vessel network and gonads. Eversion of viscera usually follows intensive constriction of the body wall. The viscera everted through the cloaca and the anus. The environmental stimuli that induce eversion include sudden increase or decrease of temperature, polluted water and physical or chemical stimulation.

Regeneration capacity is very highly developed in *S. japonicus*. When its caruncle is cut off, a small convex growth will appear at the same place in about 5–7 days and it grows to 1–2 mm in thirty days. When the tentacle is cut off, the wound heals and a convex growth appears in the same place in about 7–10 days, while the tentacle regenerates fully in 30–35 days and functions normally. Any wound up to 2–4 cm long on the ventral or dorsal side gets completely cured in 5–7 days. The regenerative capacity is stronger in the posterior region.

The regeneration in *S. japonicus*, which has everted its digestive tract and respiratory trees, proceeds as follows. The ventral central longitudinal muscle and two dorsal longitudinal muscles get connected by a suspended membrane after 5–7 days, the digestive tract is formed after 9 days, one respiratory tree is formed and the linear digestive tract is very obvious from the mouth to the total cloaca after 14 days and in about 25 days the respiratory trees develop noticeably and the digestive tract bulges. At this time, it begins to feed a little and about 33 days later, the respiratory trees resume functioning gradually.

The speed by which the digestive tract and respiratory trees of *S. japonicus* regenerate varies at different life stages. At resumption stage after aestivation, regeneration is very rapid. The digestive tract and respiratory trees regenerate and become completely functional in 25–33 days at this stage. However, such regeneration needs 8 weeks during other life stages.

1.5.2 Estivation

Generally, the period of estivation is usually from June to late September. When water temperature is at 3 °C, normal activity is hindered. When the temperature rises to 8–10 °C, the sea cucumber gets into its most active living stage. At 17–19 °C, it moves slowly and feeds less and its digestive tract begins to degenerate. At 20–25 °C, it stops feeding, discharges food in the digestive tract, stays under rock or in the crevice of a rock and begins to estivate. When temperature drops to 17–20 °C, it resuscitates, crawls out of its shelter and begins to feed.

Temperature is generally the main cause of estivation. Estivation is a means of ecological adaptation to high temperature. According to some, aestivation is resorted to recover from exhaustion caused by the discharge of genital products.

1.6 Embryology and Development

1.6.1 Reproduction

a. Gonad Development

S. japonicus is a dioecious animal, but it is difficult to distinguish sexes from outward appearance of the gonads. The colour of gonads after a closer examination is a reliable indicator. The gonad is branched. A tube, called the genital tube, extends forward from the gonad.

According to the developmental stages of the gonadal gland, five reproductive phases are distinguishable: namely spent, proliferative, active, ripe and spawning.

b. Reproduction

The reproduction of *S. japonicus* is mainly dependant on water temperature. It spawns at 15–23 °C, usually at 18–20 °C. Eggs are discharged at 2100–2400 hrs. Only rarely, it spawns after midnight or in the afternoon. The male releases sperms first and the female releases eggs later. Sperms appear milk-white in the water and eggs are orange-yellow. The female can spawn 1–3 times at an interval of 5–15 minutes or longer. Fecundity is 300,000–500,000.

1.6.2 Development

a. Embryo Development

- Sperm and egg. The mature sperm of *S. japonicus* does not move inside the testis. It moves actively only after it gets into seawater. Eggs are demersal and only have primary egg membrane, which is formed by the primary cytoplasm of egg.
- Fertilization. Eggs are fertilized in seawater.
- Cleavage. Because it is isolecithal, it cleaves completely.
- Blastula.
- Gastrula.

b. Larval Development

- Auricularia. Figure 3a. Because the larva of *S. japonicus* is ear-like in this stage, it is called auricularia and has the following structures:
 - i. Longitudinal ciliated band
 - ii. Larval arm
 - iii. Left anterior coelom sac, hydrocoel and posterior coelom. From hydrocoel, primary buccal tentacle and radial water-vessel are formed.
- Doliolaria. Figure 3b. It has the following characteristics:
 - i. Ciliated ring
 - ii. Water canal ring and Polian vesicle
 - iii. Developed posterior coelom
 - iv. Vestibule
- Pentactula. Figure 3c. It has the following characteristics:
 - i. Oral widened, the anus lost for a period and formed again. Five tentacles protrude from the vestibule.
 - ii. Ciliated ring degenerates until it disappears.
 - iii. Digestive tract is prolonged, winded; respiratory trees are going to be developed.
 - iv. Spicula is formed by primary mesenchyme.
 - v. There is a group of cells near the posterior coelom, which will develop into posterior.
- Juvenile. The first tube foot is formed under the anus on the ventral side. Its body colour varies from colourless, half transparent, light brown to dark brown.

Table 3 shows the developmental stages of *S. japonicus* after fertilization at 20–21 °C.

Table 3. At 20–21 °C, development stages of *S. japonicus* after fertilization.

| Time after fertilization | Developmental Stages | Size (•m) |
|--------------------------|---------------------------|---------------|
| 20–30 min. | Polar body appears | 150–180 |
| 43–48 min. | First cleavage (2 cells) | |
| 48–53 min. | Second cleavage (4 cells) | |
| 1 h. 30 min.-- | | |
| 2 h. 30 min. | Third cleavage (8 cells) | |
| 3 h. 40 min.-- | | |
| 5 h. 40 min. | Blastula | |
| 12–14 h. 20 min. | Hatched from egg membrane | |
| 14 h. 20 min.-- | | |
| 17 h. 40 min. | Primary stage of gastrula | |
| 17 h. 40 min.-- | | |
| 25 h. 20 min. | Later stage of gastrula | @ 500 |
| 25 h. 20 min.-- | | |
| 31 h. 30 min. | Auricularia (small) | |
| 5–6 days | Auricularia (medium) | @ 700 |
| 8–9 days | Auricularia (large) | @ 800–1,000 |
| 10 days | Doliolaria | @ 4,000–5,000 |
| 11–12 days | Juvenile | |

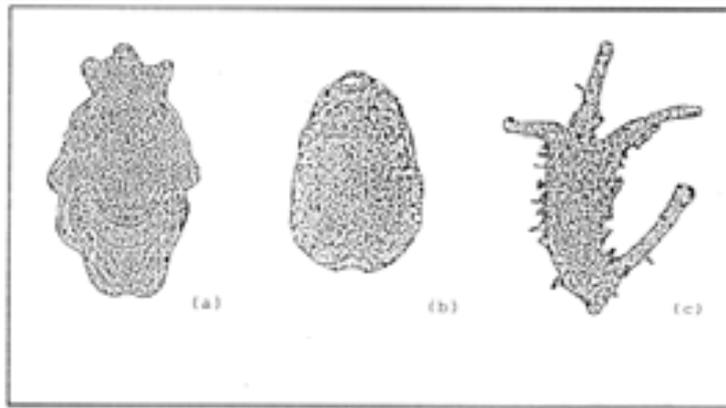


Figure 3. Three major embryological stages of sea cucumber: a) auricularia, b) doliolaria, and c) pentactula.

CHAPTER II ARTIFICIAL BREEDING OF *Stichopus japonicus*

2.1 Breeding Status of *Stichopus japonicus*

As early as 1930, Japanese scholars began to study the artificial breeding techniques of sea cucumber, *S. japonicus*. For example, in 1937, a Japanese, Inaka Densapuro, succeeded in rearing fertilized eggs to auricularia in 15 days. Until the 1950s, much useful information covering the ecological habit, life history and gonad development was increasingly gathered by Japanese biologists. These informations provided the basis for artificial breeding of sea cucumber. In 1950, Imai Taiju *et al.* were able to obtain 569 juveniles of *S. japonicus* in a 1.9 m³ tank using non-colour flagellates as larval diet. In 1977, another Japanese, Ishida Masatoshi, reported that 2.975 auricularia were obtained from induced spawning using thermal shock in the experimental farm of Fukuoka county.

In the USSR, Mokpelsba (1973) carried out artificial breeding of sea cucumber in Big Peter Bay by means of thermal shock and using *Phaeodactylum tricornutum* and *Platymonas* spp. as diet of postlarvae. However, no mention was made of the survival rate of juveniles and the report only briefly mentioned the stocking density as 4000 individuals per m².

In China, the study of sea cucumber has focussed on artificial breeding since the late 1970s, dealing with the relation of gonad development, spawning habit, embryonic development and postlarvae development to the physical, chemical and biological factors in the environment. Studies on the feeding habit at every stage of development of postlarvae, selection of diet species and other topics have provided a useful scientific basis for artificial breeding on a commercial scale.

From 1973 to 1985, the Yellow Sea Fisheries Research Institute and its cooperative units worked jointly to improve the techniques of artificial breeding of *S. japonicus*, including collection of broodstock, spawning, fertilization, rearing of postlarvae and juveniles, diet species and their cultivation method, prevention and control of natural enemies and other aspects. These studies have led to the development of suitable technologies for artificial breeding and rearing of *S. japonicus*.

2.2 Basic Facilities for Artificial Breeding

2.2.1 Nursery Room and Feeding Room

A new nursery room should be selected near the seashore where the water is calm, free of pollution, and there is no in-flow of freshwater. The nursery room should preferably face south-east, with good ventilation and uniform illumination. The optimum light intensity required in the room is 1000–2000 lux.

The ratio of area of rearing tanks of larvae and unicellular algae should be 1:4. However, in the case of high density cultivation of unicellular algae, their rearing can be decreased.

2.2.2 Broodstock and Postlarvae Rearing Tanks

Rearing tanks are the main components of the nursery room. At present, the general construction of the tanks is of brick and concrete or reinforced concrete. They should be rectangular with rounded corners and should be suitable for rearing with flowing water. The size of the rearing tank should be preferably between 5 m³ and 10 m³. The height of the tank should be less than 1 m. The required volume of the tank for *S. japonicus* broodstock is relatively small, which is about 1.5–2 m³. The intake and drain pipes should be well integrated for efficient operations.

2.2.3 Settling tank

Suspended solids in the seawater supply, such as silt, bits of organic matter and plankton should be allowed to settle, a process which generally takes more than 24 hours. The settling tank should be covered to darken the tank and hasten the settling of the plankton. The tank should also have a layer of heat insulation to prevent strong sunshine from raising water temperature. Settling tanks must be regularly cleaned to prevent the build up of harmful materials such as H₂S, NH₃ and so on. The bottom of the tanks should be swept once every 4–5 days. The total capacity of the settling tank may be 2–3 times as much as the volume of water used every day. The tank can be partitioned into several compartments, so that settling and cleaning can be rotated among the compartments.

2.2.4 Filtration tank

The water from the settling tank, after being precipitated, may be used directly in the nursery room, or filtered with a silk-bolting cloth and cotton or sand before it is piped into the nursery room.

1) Sand filtered tank: Water passes through the layer of filtering materials by gravity. The filtering materials consist of layers of different size sand and stone. Cobble, gravel, grit and fine sand are placed in layers and thickness of each layer is about 10–15 cm; the thickness of the fine sand layer should be increased appropriately. The total thickness of the filter is 40–60 cm.

2) High-pressure sand filter tank: This is a closed system, in to which water is forced into the filter tank by pump or from a highly elevated settling tank. Its construction is simple. It is built with reinforced concrete or steel plate, with a sieve plate in the centre of the tank, on which cobble stones and sand are placed (the same way as in a sand filter tank). Sometimes, fish net and window screen are spread on the sieve plate, over which are spread silk bolting cloth and then fine sand.

2.3 Gonad Developmental Stages

2.3.1 Resting Stage

Male • : In this stage, which lasts from the last ten-day period of June up to November, the gonad is small and its epithelium along the wall of the tube is without bumps and holes. The epithelium consists of three layers of spermatogonia or spermatocytes. In general, weight of the gonad is below 0.2 g.

Female • : Gonad is small and its epithelium along the wall of the tube is without bumps and holes; it consists of two layers of oocytes, with diameter of about 10 •m.

2.3.2 Recovering Stage

This stage generally lasts from December to March. The weight of the gonad generally varies from 0.2–2 g, with the gonadal index below 1 %. Gonads develop slowly, colourless or light yellow, some can now be distinguished as female or male. The diameter of oocytes is 30–50 •m; the nucleoli in nucleus are noticeable. The sperms have not been formed.

2.3.3 Developing Stage

This stage can be divided into developing stage I and developing stage II. In general, stage I is from March to the first ten-day period of May. In this stage, gonads gradually grow in thickness and branch more. Weights are generally 2–5 g and the colour apricot or light tangerine. Female and male can already be distinguished. The gonadal index is generally 1–3 %.

The stage II occurs during the second and third ten-day periods of May. In this stage, gonads develop very quickly and gain weight rapidly (generally 3–13 g). Those heavier than 7 g comprise 70 % of the total. The colour becomes darker and the index rises to about 70 %. The diameters of oocytes range from 60–90 •m, sperms have formed.

2.3.4 Ripening Stage

This stage is generally from the last ten day period of May to the first ten-day period of June. Gonads become thicker and the colour turns dark. Those heavier than 10 g comprise fifty percent of the total. The index of about one-half of the total reaches ten percent. The testes are filled with sperms; the diameter of oocytes is 100–130 μ m.

2.3.5 Spawning Stage

Releasing of eggs and sperms begins during the first ten-day period of June. Usually, gonads of bigger broodstock develop earlier, faster and mature more rapidly. Initial spawning occurs when the individuals are about 110 g in total weight and 60 g in body wall weight. The number of eggs per g of ovary varies from 220,000 to 290,000. The broodstock whose body weights range from 200 to 350 g generally have a fecundity of 2.5 m to 3.6 m eggs.

2.4 Collection of Broodstock

2.4.1 Collection Timing and Water Temperature

The key to a successful collection of well-developed broodstock is the timing and proper water temperature during collection. 15–16 °C is appropriate temperature to collect the broodstock since the rate of fluctuation of water temperature varies in different areas, there is need for measures to suit local conditions. Before the broodstock are collected, samples should be taken and dissected for observation. Early collection must be avoided. However, if they are raised too long, their gonads will atrophy and if they are collected too late they will lay eggs in the sea.

2.4.2 Broodstock Collection Standards

Measurements have shown that the following information is needed to guide one in selecting broodstock: when the weight of body wall of *S. japonicus* reaches 255 g (the weight of body wall is about one-half of that of the body), its gonad is 98 g; when the weight of body wall is 130 to 255 g their gonads are 34.7 g on an average and the mean gonadal index is 16.6 %. When the weight of body wall is from 80–110 g, the gonads are 5.6 g.

The above data indicates that the size of *S. japonicus* to be collected should be over 20 cm and should weigh over 250 g (their body wall would be about 130 g). In general, wall weight is directly related to gonad weight and gonad index to fecundity.

2.4.3 Broodstock Collection Methods

The quality of broodstock has a great bearing on breeding. When broodstock are collected, the following must be strictly observed:

- i. Collect large individuals with thick body walls and body length of over 20 cm must only be collected. The body surfaces should be free of injury.
- ii. In the course of collecting broodstock, they should not be allowed to get in touch with greasy dirt.
- iii. The broodstock collected from the sea should be put in good quality water and care should be taken to change water often and to avoid direct light.

2.4.4 Broodstock Transportation

After collection, the breeders can be transported by road or boat. Transportation by boat is preferable since it will be free from bumps, which are characteristic of land journey and which may cause injury to the breeders. If the breeders have to be transported by road, they should be put in a plastic bag containing seawater and the bag placed in a canvas bucket filled with seawater. The bucket should be covered with some algae to keep seawater from spilling out and to shut out light. The best time for transportation is early morning or evening.

2.5 Broodstock Rearing

2.5.1 Rearing Density

If density of broodstock is too high, it will result in rapid depletion of dissolved oxygen. An environment of low DO over a long period causes abnormal behavior in the broodstock in that they tend to crimp their bodies and creep incessantly on the surface of the tank wall. It also adversely affects gonadal development, resulting in failure to release the sexual products normally. When the density of breeders is more than 40 individuals per m³, 70 percent of them have been observed to fall to the tank bottom and become stiff, an indication of paralysis, because of dissolved oxygen level dropping to 0.6 mg/l. When the density of breeders is 30 individuals per m³, the DO level is generally below 5–6 mg/l. Therefore, it is desirable to maintain the broodstock at 20–30 individuals per m³.

2.5.2 Broodstock Management

- i. For the water in the raising tank to remain fresh and have normal DO, one half or one-third of the volume should be changed once in the morning and again at night.
- ii. Excreta and dirt in the tank should be removed immediately.
- iii. The behavior of the individual breeders should be constantly watched.

The discharge of sperms by some of the males and the frequent trips of the females to the tank wall where they hold their head high and keep swaying, are indications that the breeders are ready to spawn. It is necessary to make all the preparations for spawning and further care well in time.

2.6 Spawning and Handling of Eggs of Embryos

2.6.1 Spawning

The main aim of artificial breeding is to successfully obtain high quality zygotes. Natural spawning and the various methods for inducing spawning are detailed below.

i. Natural spawning

When their gonads are fully mature, the male and female breeders release their gametes naturally, without any inducements. At first, the male releases the sperms, which induces the female to release eggs after about half an hour. The eggs are generally released around 2000–2100 hrs. Some females are able to release eggs continuously for more than 45 mins. One female can release more than 1 million eggs at one time and a total of 4–5 million eggs during one spawning phase.

ii. Stripping

This method was used by a Japanese (Inaka Densapuro) during the 1930s and a Chinese (Fengying Zhang) in the 1950s. The rate of fertilization is as low as 20% and the number of deformed individuals is large. In this method, to start with the back of the breeder is cut open with a scissor from anus upwards. The ovary or testis is taken out and dried in the shade. The ovary is then placed in a container filled with seawater and torn lightly with tweezers or scissors to release the eggs into the seawater. The eggs are filtered off from the water with a gauze and set aside. The testis is placed in another container with seawater and cut to pieces, when the sperms swim out into the seawater. The seawater with eggs in then poured into the one with sperms for the eggs to be fertilized. It is difficult to get a high rate of fertilization with this method and is, therefore, limited only to small-scale experiments.

iii. Thermal shock

This method is often used to induce spawning in marine invertebrates, such as molluscs and echinoderms. The water temperature of filtered seawater can be raised by exposure to intense sunshine, or with an electric heating rod, or by adding hot water with a temperature higher by 3–5 °C than that of the filtered seawater. This thermal shock stimulates the breeders to discharge sperms or eggs. This method is widely used.

iv. Stimulation through desiccation and flowing water

This method can be used after the breeders have been conditioned over 7–10 days. Stimulation for inducing spawning is generally carried out at dusk. First, the tank is emptied of all the water and the broodstock left to dry in the shade for a period of time. They are then subjected to high pressure seawater for several minutes. While applying water pressure, the tank should be scrubbed clean and later filled with filtered seawater. After the breeders have been stimulated for 1.5–2 hours, they begin to move up the tank wall and move about frequently. First, the male will release sperms in about half an hour's time after which, the female is induced to release eggs. This method can generally result in 95–100 % fertilization. With this method, one can plan in advance and work out a programme for use of facilities, food propagation, and other breeding operations.

2.6.2 Fertilization

It is important to ensure a high survival rate in artificial breeding by obtaining high quality eggs. Therefore, it is necessary to handle the eggs carefully as soon as they are released. Two procedures are followed in this regard at present.

- i. After they have released their eggs or sperms, the breeders are removed from the tank. The eggs are washed several times, usually in the early morning hours of the day after spawning. However, this method is not very satisfactory, since the large quantity of sperms released into the same tank might pollute the water, resulting in reduced fertilization and a large number of deformed embryos.
- ii. The second procedure involves the use of an egg-box for keeping the eggs separately. When the high peak of spawning begins, constant observation of the broodstock is needed. A person must be assigned to conduct inspection tours in the evening. As soon as the male is observed to release sperms, the inspection tours to the tanks should be done more frequently. When the female breeders begin to release eggs in large numbers, they should be gently moved to a specially prepared "eggs-box" holding filtered seawater to continue spawning. Because the eggs of *S. japonicus* tend to sink the water must be stirred with a glass rod as the eggs are being released in order to keep the eggs suspended so as to increase fertilization rate. After the releasing of eggs is completed, the breeders must be moved out at once and the water stirred thoroughly. A sample is taken to estimate the number of eggs and the status of fertilization. In general, matured high quality eggs are spherical and evenly formed. Their diameter is generally about 140–170 μm , while the length of normal sperm head is 6 μm . After fertilization, the zygotes should be transferred with a rubber pipette into a rearing tank, which has been washed clean and contains filtered seawater. The density of eggs put in postlarval rearing tank is 10 million per m^3 . While the eggs are being piped into the rearing tank, filtered seawater is gradually added so that the eggs are distributed uniformly. This helps to improve the rate of fertilization, which should be aimed at 95–100%.

The density of eggs in the eggs-box should be kept below 100 per ml.

CHAPTER III REARING OF POSTLARVAE

3.1 Preparation of Rearing Tanks

Rearing tanks and other tanks used in breeding, especially the new tanks, must be scrubbed clean and then kept filled with water for 25 to 30

days, during which period the water is changed repeatedly in order to lower the pH to less than 8.5. Before the tanks are used, they are scrubbed and filled with water containing 40 ppm bleaching powder and then washed clean with filtered seawater before the larvae are introduced.

3.2 Rearing Density

Strict control of rearing density of larvae, i.e. the number of larvae per ml of water, is required. At present, there are two methods to rear the larvae: still water rearing and flowing water rearing. Auricularia, during their early and middle stages, concentrate at the surface of the water. If the density of larvae is too high, they will cohere into a ball and sink, resulting in death. Therefore, controlling rearing density would ensure a better survival rate. The Yellow Sea Fisheries Research Institute, the Marine Fisheries Research Institute of Liaoning Province, and the Marine Fisheries Research Institute of Shandong Province have separately carried out several experiments on rearing density. The results of the experiments indicate that the desirable density is 300–700 postlarvae per litre.

3.3 Selection and Counting of Larvae

After the fertilized eggs are moved to the rearing tank, they develop into the early auricularia stage in about 30-hours. The bottom of rearing tanks should be cleaned completely. Healthy larvae occupy the surface layer of water, while the deformed larvae and dead embryos generally stay in the lower layer of the water column or at the tank bottom. All the dead individuals, deformed larvae and sediment should be siphoned out in order to clean the tanks. After the tanks are cleaned, the rearing water is stirred lightly up and down for the larvae to be evenly distributed. A sample is then taken for counting of larvae. Samples are taken separately from the two ends and the middle of the tank with a 250 ml beaker; in turn three smaller samples are obtained from the first sample with a small cylinder or measuring pipette. These are used for counting. The average of three counts is taken as an indication of the density of larvae. The result of the count would show whether the density is desirable or not. When auricularia are in their early stage, they are reared at a density level of about 500 per litre. The period of auricularia development can be divided to three stages: early, middle and late stages. As they develop from one stage to the next, the bottom of tanks must be cleaned completely once, or the larvae moved to another tank. The sediment must be removed to keep the water fresh. An up-to-date information on the survival rate at each developing stage is necessary.

3.4 Water Management

In the course of rearing, the larvae eject faeces and consume dissolved oxygen constantly. Some of the larvae die in course of time. These and the leftover food produce harmful substances, such as H_2S , and NH_3 . In addition, bacteria reproduce rapidly with rise in temperature. Poor water quality directly affects the normal development of larvae. Therefore, proper water management and sanitation is essential. Cleaning of tanks and changing water are essential. There are several methods of changing water. In the still-water rearing method, which is used in some places, the tank is gradually filled with water during the earlier stage of rearing. The dirt and deformed larvae on the tank bottom are siphoned out every day. Net cages are used for changing water. Attention must be paid to the mesh size of the silk-bolting cloth of net cages; the mesh size must be smaller than the body width of larvae, otherwise they will be washed away. While the water is being changed with net cage, someone should constantly stir the water lightly all around the tank. This will prevent the loss of larvae during water change, since siphoning would normally force the larvae to stick to the net cages, which might cause mechanical injury to the larvae. The sediments on the bottom of tanks should be siphoned out completely every three or four days.

Since 1982, the Yellow Sea Fisheries Research Institute has used the method of rearing in flowing water, which maintains a better water quality and avoids injury to larvae. The water flow in a $7m^3$ tank should be maintained at 6,000 ml/min. With the flow at this volume for 8–10 hrs every day, the quantity of water that is changed is more than half of the total volume. The water flow should be stopped for one hour while food is being put in.

3.5 Food Species and Feeding Rates

Suitable and high quality species of diet and appropriate feeding schedule are the key to successful rearing.

As the larva of *S. japonicus* develops into early auricularia stage, its alimentary canal is linked up and the larva must be given its diet at once. The feeding mechanism of the larvae consists of conveying the suspended bits of organisms and unicellular algae into the alimentary canal through the mouthparts by the swaying of the peristomial cilia. The effectiveness of several unicellular algae as larval diet has been studied. The results show that *Platymonas*, even though it can be easily bred on a large scale, cannot be ingested by larvae because of its big size and powerful moving ability. In addition, the larvae cannot easily digest the thick cell wall of *Platymonas*. Thus, the larvae fed on this species develop slowly, have low survival rate, and more deformed individuals. *Dunaliella* sp. is effective, since it has no cell wall and can be easily digested. Hence, the larvae develop quickly and show high survival rate. *Phaedocatylum tricornutum* is small in size, has weak moving ability and can be easily propagated on a large scale. Its effectiveness as a diet is also very good. A notable point in using this diet is that the optimum temperature for its reproduction and growth is 14–18 °C, which is identical with the temperature requirement of the early part of larval development. *Dicratetaria* sp., whose temperature requirement for reproduction and growth is 18–28 °C, can meet the diet requirement of the later part of larval. Some also use *Chaetoceros* sp. and *Isochrysis* sp.

The larvae require different quantities of diet during different development stages. Unicellular algae are fed twice a day, but the quantity given each time depends on the particular stage of the larvae. In general, it is from 19,000–30,000 cells per ml of rearing tank water. The quantity of diet given should be increased or decreased depending on the quantity of food in the stomach of sea cucumber, which has to be checked every day. Unicellular algae during the peak period of their reproduction are the most preferred diet for the larvae. Combinations of a few algae are more effective than a single species diet.

3.6 Environmental Factors

3.6.1 Temperature

The optimum temperature for rearing larvae of *S. japonicus* is 18–22 °C. The water temperature should be measured twice a day, in the morning and afternoon.

3.6.2 Dissolved Oxygen

Dissolved oxygen level varies with water temperature. The higher the temperature, the lower the DO level. Two units are used for DO level, viz. ml per l, and mg per l and their conversion relation is as follows:

$$1 \text{ mg/l} = 0.7 \text{ ml/l}, \text{ or } 1 \text{ ml/l} = 1.43 \text{ mg/l}$$

3.6.3 pH

Under normal conditions, the rearing seawater is generally alkaline with a pH of 7.5–8.6. Tests have shown that *S. japonicus* larvae and juveniles adapt to a fairly wide range of pH. However, when pH rises over 9.0 or drops below 6.0, the moving ability of the larvae weakens and growth stops. Therefore, pH value of the water must be kept between 6.0 and 9.0.

3.6.4 Salinity

The salinity of normal seawater is 32–34 ppt. If the temperature of the rearing water is 18–22 °C and salinity 1.5–12.9 ppt all the larvae will die in 1–2 days. The larvae reared in 19 ppt salinity seawater for 4 days stop developing further. In 26.2–32.7 ppt salinity, they develop normally, but in 39.3 ppt salinity they develop slowly and their size remains small. The lethal critical salinity is 12.9 ppt. The optimum salinity for larval development ranges from 26.2–32.7 ppt. In this range, higher the salinity, quicker is their development. Both too high and too low salinity values adversely affect the normal development of embryo and larvae, resulting in a large number of deformed individuals or even causing death. Salinity measurement is, therefore an important routine work throughout the entire rearing period. A salinity refractometer is now commonly used for salinity measurement. If a specific gravity meter is used, the measured value can be converted into salinity value using the following formula or by consulting the salinity-specific gravity index chart (Table 4):

- a. When the water temperature is over 17.5 °C,

$$S(\%) = 1305 (\text{specific gravity} - 1) + (t - 17.5) \times 0.3.$$

- b. When the water temperature is below 17.5 °C,

$$S(\%) = 1305 (\text{specific gravity} - 1) - (17.5 - t) \times 0.2.$$

3.6.5 Ammoniacal Nitrogen

The ammoniacal nitrogen content of seawater is very low. Its sources in breeding tanks are mainly the metabolites of the larvae, the unconsumed diet and decomposing organisms. Too much accumulation of NH₃ can be harmful to the larvae. The larvae can develop normally with an ammoniacal nitrogen content of 70–430 mg per m³ water. When its content is over 500 mg per m³, it will have a harmful effect on the development and growth of larvae.

Table 4. Salinity-specific gravity index chart.

| * a _t S _{1/11} t °C | 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.0 | 15.0 | 16.0 | 17.0 | 18.0 | 19.0 | 20.0 | 21.0 | 22.0 | 23.0 | 24.0 | 25.0 | 26.0 | 27.0 | 28.0 | 29.0 | 30.0 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 0 | | | | 2.7 | 4.0 | 6.2 | 0.4 | 7.7 | 8.0 | 10.2 | 11.3 | 12.7 | 13.8 | 15.0 | 16.3 | 17.5 | 18.8 | 20.0 | 21.3 | 22.5 | 23.8 | 26.0 | 26.3 | 27.5 | 28.8 | 30.0 | 31.0 | 32.6 | 33.8 | 35.0 | 36.1 |
| 1 | | | | 2.0 | 3.0 | 5.1 | 6.5 | 7.6 | 8.8 | 10.1 | 11.3 | 12.0 | 13.8 | 15.0 | 16.3 | 17.5 | 18.8 | 20.1 | 21.3 | 22.5 | 23.8 | 25.0 | 26.3 | 27.5 | 28.8 | 30.0 | 31.3 | 32.6 | 33.8 | 35.1 | 36.2 |
| 2 | | | | 2.4 | 3.7 | 5.1 | 6.2 | 7.5 | 8.8 | 10.0 | 11.3 | 12.0 | 13.8 | 15.0 | 16.3 | 17.5 | 18.8 | 20.1 | 21.3 | 22.5 | 23.8 | 25.0 | 26.3 | 27.6 | 28.8 | 30.1 | 31.3 | 32.6 | 33.8 | 35.1 | 36.3 |
| 3 | | | | 2.4 | 3.7 | 5.1 | 6.2 | 7.0 | 8.8 | 10.0 | 11.2 | 12.9 | 13.8 | 15.0 | 16.3 | 17.5 | 18.8 | 20.1 | 21.3 | 22.6 | 23.9 | 25.1 | 26.4 | 27.6 | 28.9 | 30.2 | 31.4 | 32.7 | 33.9 | 35.2 | 36.1 |
| 4 | | | | 2.4 | 3.7 | 5.1 | 6.2 | 7.9 | 8.8 | 10.0 | 11.2 | 12.0 | 13.8 | 15.0 | 16.3 | 17.5 | 18.8 | 20.1 | 21.3 | 22.6 | 24.0 | 25.1 | 26.5 | 27.6 | 28.9 | 30.3 | 31.4 | 32.7 | 34.0 | 35.2 | 36.6 |
| 5 | | | | 2.4 | 3.7 | 5.1 | 6.2 | 7.5 | 8.8 | 10.0 | 11.2 | 12.0 | 13.8 | 15.0 | 16.4 | 17.6 | 18.9 | 20.2 | 21.4 | 22.7 | 24.1 | 25.2 | 26.6 | 27.8 | 29.0 | 30.3 | 31.6 | 32.8 | 34.1 | 35.4 | 36.7 |
| 6 | | | | 2.4 | 3.7 | 5.1 | 6.2 | 7.5 | 8.8 | 10.0 | 11.2 | 12.7 | 13.8 | 15.1 | 16.5 | 17.7 | 19.0 | 20.3 | 21.6 | 22.8 | 24.1 | 25.3 | 26.6 | 27.9 | 29.1 | 30.4 | 31.7 | 33.0 | 34.2 | 35.5 | 36.8 |
| 7 | | | | 2.5 | 3.8 | 5.1 | 6.3 | 7.6 | 8.9 | 10.1 | 11.4 | 12.7 | 13.9 | 15.2 | 16.5 | 17.8 | 19.0 | 20.3 | 21.6 | 22.9 | 24.1 | 25.4 | 26.7 | 28.1 | 29.2 | 30.6 | 31.8 | 33.2 | 34.2 | 35.6 | 36.9 |
| 8 | | | | 2.6 | 3.9 | 5.1 | 6.4 | 7.7 | 9.0 | 10.2 | 11.6 | 12.8 | 14.0 | 15.3 | 16.6 | 17.9 | 19.1 | 20.4 | 21.7 | 23.0 | 24.2 | 25.6 | 26.8 | 28.2 | 29.3 | 30.6 | 31.9 | 33.3 | 34.4 | 35.7 | 37.0 |
| 9 | | | | 2.6 | 3.9 | 5.2 | 6.5 | 7.7 | 9.0 | 10.3 | 11.6 | 12.8 | 14.1 | 15.4 | 16.8 | 18.1 | 19.2 | 20.6 | 21.9 | 23.2 | 24.4 | 25.7 | 27.0 | 28.3 | 29.5 | 30.8 | 32.1 | 33.4 | 34.6 | 35.9 | 37.2 |
| 10 | | | | 2.7 | 4.0 | 5.3 | 6.6 | 7.8 | 9.1 | 10.4 | 11.7 | 12.9 | 14.2 | 15.5 | 16.9 | 18.2 | 19.4 | 20.7 | 22.0 | 23.3 | 24.6 | 25.8 | 27.1 | 28.4 | 29.7 | 31.0 | 32.3 | 33.6 | 34.8 | 36.1 | 37.4 |
| 11 | | | | 2.9 | 4.2 | 5.4 | 6.7 | 8.0 | 9.3 | 10.6 | 11.9 | 13.1 | 14.4 | 15.7 | 17.0 | 18.5 | 19.6 | 20.9 | 22.2 | 23.5 | 24.8 | 26.0 | 27.3 | 28.6 | 29.9 | 31.2 | 32.5 | 33.8 | 35.0 | 36.2 | 37.6 |
| 12 | | | | 3.0 | 4.3 | 5.5 | 6.8 | 8.1 | 9.4 | 10.7 | 12.0 | 13.2 | 14.5 | 15.8 | 17.1 | 18.4 | 19.7 | 21.1 | 22.4 | 23.7 | 24.9 | 26.2 | 27.5 | 28.8 | 30.1 | 31.4 | 32.7 | 34.0 | 35.2 | 36.6 | 37.8 |
| 13 | | | | 3.1 | 4.4 | 5.7 | 7.0 | 8.3 | 9.6 | 10.9 | 12.2 | 13.4 | 14.7 | 16.0 | 17.3 | 18.6 | 19.9 | 21.3 | 22.6 | 23.9 | 25.1 | 26.4 | 27.7 | 29.0 | 30.3 | 31.6 | 32.9 | 34.2 | 35.5 | 36.8 | 38.1 |
| 14 | | | | 3.3 | 4.6 | 5.9 | 7.2 | 8.5 | 9.8 | 11.1 | 12.4 | 13.6 | 14.9 | 16.2 | 17.6 | 18.8 | 20.1 | 21.5 | 22.8 | 24.1 | 25.3 | 26.6 | 27.8 | 29.2 | 30.5 | 31.8 | 33.4 | 34.4 | 35.7 | 37.0 | 38.4 |
| 15 | | | 2.0 | 3.4 | 4.7 | 6.0 | 7.3 | 8.6 | 9.9 | 11.2 | 12.5 | 13.8 | 15.1 | 16.4 | 17.7 | 19.0 | 20.2 | 21.7 | 23.0 | 24.3 | 25.5 | 26.8 | 28.1 | 29.4 | 30.7 | 32.0 | 33.4 | 34.7 | 36.0 | 37.2 | 38.7 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 16 | | | 2.3 | 3.6 | 4.9 | 6.2 | 7.5 | 8.8 | 10.1 | 11.4 | 12.7 | 14.0 | 15.3 | 16.9 | 17.9 | 19.2 | 20.5 | 21.9 | 23.2 | 24.5 | 25.8 | 27.1 | 28.4 | 29.7 | 31.0 | 32.3 | 33.7 | 35.0 | 36.3 | 37.6 | 38.9 |
| 17 | | | 2.5 | 3.7 | 5.1 | 6.4 | 7.7 | 9.0 | 10.3 | 11.6 | 12.9 | 14.2 | 15.5 | 16.9 | 18.2 | 19.5 | 20.8 | 22.1 | 23.4 | 24.7 | 26.1 | 27.4 | 28.7 | 30.0 | 31.3 | 32.6 | 33.9 | 35.2 | 36.5 | 37.8 | 39.2 |
| 18 | | | 2.8 | 4.0 | 5.4 | 6.7 | 8.0 | 9.3 | 10.6 | 11.9 | 13.2 | 14.4 | 15.7 | 17.1 | 18.4 | 19.7 | 21.0 | 22.3 | 23.6 | 24.9 | 26.3 | 27.6 | 28.9 | 30.2 | 31.5 | 32.8 | 34.1 | 35.4 | 36.8 | 38.2 | 40.5 |
| 19 | | | 3.0 | 4.3 | 5.6 | 6.9 | 8.2 | 9.5 | 10.8 | 12.1 | 13.4 | 14.7 | 16.0 | 17.3 | 18.6 | 19.9 | 21.3 | 22.6 | 23.9 | 25.2 | 26.6 | 27.9 | 29.3 | 30.5 | 31.8 | 33.1 | 34.4 | 35.7 | 37.1 | 38.5 | 38.8 |
| 20 | | 1.8 | 3.2 | 4.5 | 5.8 | 7.2 | 8.5 | 9.8 | 11.1 | 12.4 | 13.7 | 15.0 | 16.3 | 17.6 | 18.9 | 20.2 | 21.6 | 22.9 | 24.2 | 25.5 | 26.9 | 28.2 | 29.6 | 30.8 | 32.1 | 33.4 | 34.7 | 36.0 | 37.4 | 38.8 | 40.1 |
| 21 | | 2.1 | 3.4 | 4.7 | 6.1 | 7.4 | 8.7 | 10.0 | 11.3 | 12.7 | 14.0 | 15.3 | 16.0 | 17.9 | 19.2 | 20.6 | 21.9 | 23.3 | 24.6 | 25.9 | 27.2 | 28.6 | 29.9 | 31.2 | 32.4 | 33.8 | 35.1 | 36.4 | 37.7 | 39.1 | 40.4 |
| 22 | | 2.4 | 3.7 | 5.0 | 6.4 | 7.7 | 9.0 | 10.3 | 11.0 | 13.0 | 14.3 | 15.6 | 17.0 | 18.3 | 19.6 | 20.9 | 22.3 | 23.6 | 25.0 | 26.3 | 27.6 | 28.9 | 30.2 | 31.5 | 32.8 | 34.1 | 35.4 | 36.8 | 38.1 | 39.6 | 40.8 |
| 23 | | 2.7 | 4.0 | 5.3 | 6.6 | 7.9 | 9.2 | 10.0 | 11.9 | 13.3 | 14.6 | 15.9 | 17.3 | 18.6 | 19.9 | 21.2 | 22.6 | 23.8 | 25.3 | 26.6 | 27.9 | 29.2 | 30.5 | 31.8 | 33.1 | 34.4 | 35.7 | 37.2 | 38.5 | 39.8 | 41.1 |
| 24 | | 2.9 | 4.3 | 5.6 | 7.0 | 8.3 | 9.6 | 10.0 | 12.2 | 13.6 | 15.0 | 16.3 | 17.6 | 18.9 | 20.2 | 21.6 | 22.9 | 24.2 | 25.6 | 26.9 | 28.3 | 29.6 | 30.9 | 32.2 | 33.5 | 34.8 | 36.1 | 37.6 | 38.8 | 40.1 | 41.2 |
| 25 | 1.9 | 3.2 | 4.6 | 5.8 | 7.5 | 8.6 | 9.9 | 11.2 | 12.9 | 13.8 | 15.3 | 16.6 | 17.9 | 19.2 | 20.5 | 21.8 | 23.3 | 24.6 | 25.9 | 27.2 | 28.6 | 29.9 | 31.2 | 32.6 | 33.9 | 35.2 | 36.5 | 37.8 | 39.1 | 40.4 | |
| 26 | 2.3 | 3.6 | 4.9 | 6.2 | 7.6 | 8.9 | 10.2 | 11.6 | 12.9 | 14.2 | 15.6 | 17.0 | 18.3 | 19.6 | 20.9 | 22.3 | 23.7 | 25.0 | 26.3 | 27.0 | 29.0 | 30.3 | 31.6 | 33.0 | 34.3 | 35.6 | 36.9 | 38.2 | 39.5 | 40.8 | |
| 27 | 2.6 | 3.9 | 5.2 | 6.6 | 7.9 | 9.2 | 10.6 | 11.9 | 13.3 | 14.6 | 15.9 | 17.3 | 18.6 | 20.0 | 21.3 | 22.6 | 24.0 | 25.3 | 26.6 | 28.0 | 29.3 | 30.6 | 31.9 | 33.3 | 34.6 | 36.0 | 37.3 | 38.6 | 39.9 | 41.2 | |
| 28 | 2.9 | 4.2 | 5.6 | 7.0 | 8.3 | 9.6 | 11.0 | 12.3 | 13.7 | 15.0 | 16.3 | 17.7 | 19.0 | 20.4 | 21.7 | 23.0 | 24.4 | 25.7 | 27.0 | 28.4 | 29.7 | 31.0 | 32.3 | 33.7 | 35.1 | 36.4 | 37.7 | 39.0 | 40.3 | | |
| 29 | 3.7 | 4.7 | 6.0 | 7.3 | 8.6 | 10.0 | 11.3 | 12.6 | 14.0 | 15.4 | 16.7 | 18.0 | 19.4 | 20.7 | 22.1 | 23.4 | 24.7 | 26.1 | 27.4 | 28.8 | 30.1 | 31.4 | 32.7 | 34.0 | 35.5 | 36.8 | 38.1 | 39.4 | 40.7 | | |

* $a_t = (\text{reading} - 1) \times 1000$

CHAPTER IV REARING OF JUVENILES

4.1 Types of Settling Bases

When the larvae develop to the late auricularia stage, their bodies contract to half their original length. When they begin to develop into doliolaria, the shelf of settling base should be placed in time. Different units or institutes use different types of settling plate bases. For example, the Yellow Sea Fisheries Research Institute uses a 60×60×80 cm frame welded with a 0.6 cm diameter hard plastic tube. The other types used elsewhere are i) silk bolting cloth and transparent plastic film tied to the frame at 45° angle to each other, ii) No. 8 zinc plated iron wire covered with a plastic tube, the two ends of which are sealed to form the frame, iii) wooden frame, to the middle of which are inserted plates of polyethylene or some other material, iv) tiles hung in the tank and v) stones of different size placed at the bottom of the tank.

The settling bases should have the following characteristics:

- i. Juvenile *S. japonicus* that settle on them can be observed, handled and managed conveniently.
- ii. Have no toxicity to juveniles and do not spoil water quality.
- iii. Allow maximum settling of juveniles per unit area.
- iv. Easily available and inexpensive.

The size of settling base depends on the specific situation of each unit and cannot be set rigidly. In addition, the settling bases should be covered with a layer of diatoms, so that as soon as the juveniles settle on the bases, they can have ready food.

4.2 Diet of Juveniles

Just after completing their metamorphosis, the juveniles have only weak moving ability and their tentacles are short. If food cannot be provided on time, they would die. Studies have been made on the type and composition of diets appropriate for the juveniles. The seaweeds tried for this purpose include *Sargassum thumbergii*, *S. kjellmanianam*, *Pelvetia siliquosa*, *Laminaria japonica*, *Undaria pinnatifida* and *Ulva lactuca*. The seaweed was ground with seawater and the liquid filtered off through a silk-bolting cloth was mixed with the filtered liquid of ground sea grass and fed to the juveniles. Of all the diets tried, *S. thumbergii* gave high survival rate and faster growth. Juveniles with body length shorter than 2 mm take mainly benthic diatoms as diet. Unicellular algae and filtered liquid of ground *S. thumbergii* should also be fed every day. When the body length of the juveniles reaches 2–5 mm, their diet should mainly consist of filtered liquid of *S. thumbergii*, to be fed twice every day. The quantity of diet is increased daily as the juveniles grow.

4.3 Density of Settled Juvenile

When the larvae develop to the juvenile stage, they begin to crawl. Most of them stay on the settling bases. About 15 days after they have settled on the bases, they can be seen by the naked eye. They should now be counted. A random sample is taken with a 5 cm² or 10 cm² counting frame. The sampling area of each tank must be over 5 % of its total area.

In order to achieve increased survival rate, it is necessary to control appropriately the settling density on settling bases to kept it at the optimum level. Tests have shown that too thick density of settling and insufficient diet will be adverse to growth and survival. Hence, after they are counted, their density should be adjusted to the optimum, which is 200–500 individuals per m².

4.4 Juvenile Rearing in Flowing Water

The flowing water method to rear juvenile is beneficial in two ways: it keeps the water fresh and enhances the growth of benthic diatoms on setting bases. The water flow can be appropriately adjusted to about 8–10 l/min. Still water rearing requires a larger volume of water changes, especially in juvenile rearing. Change of water is critical during this stage, because of the need to maintain good water quality. Adequate diet and optimum temperature are other important requirements. The juvenile rearing period coincides with the high temperature

period.

Individual juveniles begin to attain different sizes. Since larger individuals will monopolise feed utilization, the juveniles should be segregated by size for the smaller and weaker juveniles to develop properly. The bigger individuals must be taken out and placed in a separate tank. When the juveniles have grown to a certain size, they are transferred to the sea for further growth.

CHAPTER V PREDATORS AND THEIR CONTROL

5.1 Predation

The juveniles begin to settle from the last ten days of June to July. This is also the period of high water temperature, when predators such as harpacticoids and other copepods are at the peak of their reproduction. These do much harm to juveniles that are smaller than 0.2–0.5 mm. Harpacticoids harm the juveniles by:

- i. Reproducing very rapidly in rearing tank and competing for food with the juvenile. (Unicellular algae and *Sargassum thumbergii* have been found in their alimentary canals).
- ii. Wounding the body surface of the juveniles with their mouth parts and tearing the epidermis of the juveniles, exposing the bone plates and making them vulnerable to further predation. The parasites eat away at the juveniles until they die. The infested juveniles assume a ball shape and die gradually.

5.2 Predator Control

Control trials on harpacticoids and other copepoda with different chemicals at different concentrations have been conducted. Harpacticoids are sensitive to organic phosphorus. Thus, dipterex, kogor and other chemicals containing organo-phosphorus have been tested. The results show that all harpacticoids can be killed with 2 ppm dipterex in two hours with no harmful effects on the juveniles. However, it is necessary to give careful attention to the preparation of dipterex solution of appropriate concentration. The solution is evenly sprinkled into the tank and the water in the tank must be changed completely after two hours.

PUBLICATIONS AND DOCUMENTS OF THE REGIONAL SEAFARMING DEVELOPMENT AND DEMONSTRATION PROJECT RAS/90/002 (RAS/86/024)

Working Papers RAS/86/024

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Audio-visual Materials
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Culture of the Pacific Oyster (Crassostrea gigas) in the Republic of Korea. 71 slides.

Culture of the seabass (Lates calcarifer) in Thailand. 40 slides.

Marine finfish netcage culture in Singapore. 37 slides.

Culture of Kelp (Laminaria japonica) in China. 30 minutes video.

Audio-visual Materials
RAS/90/002

Artificial breeding and culture of abalone in Korea (DPR). 60 minutes video.

