ARTIFICIAL BREEDING OF OYSTERS IN TANKS.

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By

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Introduction

An attempt to rear oyster larvae in tanks in order to obtain the spat on a commercial scale is not new in the history of oyster culture. But in only a few cases has it been possible to claim success in any degree. Among them, we can cite the works of H. A. Cole (1936) and E. Hughes (1940). Cole reported the cases of successful rearing of oyster larvae in large tanks at Conway. After several years of experiments, he arrived at the conclusion that the essential factor for tank culture at Conway was the character of food organisms rather than the physical and chemical conditions of the tank water. He enriched the tank water with crab meat in order to promote the growth of food organisms. According to his observation, the oyster larvae can utilize only minute naked flagellates belonging to the groups *Chlamydomonadineae, Cryptomonadineae* and *Chrysomonadineae* in their free swimming stage. As to the types of flagellates, there seems to be no doubt that his attention was focused on the colored type of naked flagellates. Hughes reported successful breeding at Yealm, in much smaller tanks than those at Conway (1940). He also enriched the tank water with crab meat, but no particular account was given concerning the food organisms in it. On the nature of the diet of oyster larvae, the work by Bruce *et al* (1940) is considered the most elaborate. They prepared small vessels with running water, where oyster larvae were fed on pure cultures of minute colored flagellates under carefully controlled conditions. Thus they demonstrated the suitability of some of the flagellate cultures as diet of oyster larvae. These experiments, as well as many others on the nature of the diet of oyster larvae, seemed to focus attention on the colored type of algae.

In 1941 we isolated a naked non-colored flagellate, identified as *Monas* sp., from the water of a natural seed-oyster farm at Mangoku-ura and found it

* Contribution from Onagawa Fisheries Laboratory, Tohoku University.

a favorite food for the larvae of Japanese common oyster, *Ostrea gigas*. The larvae were reared in small vessels where water was kept stagnant and not renewed during the experiment (Imai T. & M. Hatanaka, 1949). This type of flagellate differed from the colored one in its mode of nutrition, for it grew up not by photosynthesis but by ingesting the bacterial cells which multiplied during the decomposing process of organic matters in sea water. A study on nutritional requirementt in *Monas* cultures proved that the high density of the culture could be obtained easily in a medium favorable for feeding simply by enriching the sea water with a carbohydrate, such as glucose for example, as energy and carbon source and inorganic salts of nitrogen and phosphorus as nutrients (Imai T. & M. Hatanaka, 1950).

Ecological survey of Mangoku-ura, a natural oyster farm, revealed the fact that the naked non-colored flagellate was very common in coastal waters, particularly in brackish water, that it was playing an important role in the natural production of seed oysters, and further more that the abundant supply of flagellate in Mangoku-ura was kept up by docomposition of a vast amount of eel-grass, *Zostera marina*, during the breeding season of oysters (Imai T., M. Hatanaka & R. Sato, in preparation).

The utility of *Monas* sp. as a larval diet is very common among bivalves and echinoderms, as we have shown in our successful breedings of clams, *Mactra sachalinensis* (1942_1), *Meretrix meretrix* and *Venerupis philippinarum* (1942_2), a scallop, *Chlamys farreri akazara* (1944_1), a wood-borer, *Teredo navalis* (1944_2) and a sea cucumber, *Stichopus japonicus* (1942_3).

With cultures of favorite food organisms at hand, it was our great concern to apply the culture method to a large scale production of oyster spat in tanks. Essential factors for tank culture were a continuous supply of suitable food organisms and the maintenance of favorable water condition for larval growth. In the preliminary breeding experiments performed in 1943, a good supply of *Monas* sp. could be continuously obtained by enrichment of tank water with a small amount of starch, without any serious drawback in tank water for larval culture. As a result, a large amount of spat were collected (Imai T. & M. Hatanaka, 1944_3). Since then, efforts have been made in order to improve the method of culture so that uniform results might be obtained in spat production. The reports on breeding expreiments previous to 1949 were read in the annual meetings of Japanese Society of Scientific Fisheries in 1946, '47, '48 and 1949. Because of the shortage of space, the results here reported are only of the breeding experiments carried on during the season of 1949. But they will suffice to illustrate the present stage of development of our culture method.

From the standpoint of the fishing industry, the status quo of seed oyster

production in Japan does not find any need for adopting the method of artificial propagation of seed oyster on a commercial scale, because it is produced abundantly on a natural farm. Much of the seed oysters produced in Matsushima Bay and Mangoku-ura are exported to the Pacific coasts of the United States of America. Besides, Hiroshima and Kumamoto produce a considerable amount of seeds in southern parts of Japan. The study of artificial propagation, nevertheless, can find meaning in biological rasearch which will be referred to soon, and also in its application to the production of such economically valuable seeds as those of the pearl oyster.

The artificial breeding provides us means to study the life history of an animal and other biological phenomena connected with its propagation. When the ecological details of seed production are made experimentally clear, the principle will guide the management of natural seed production. Our next concern is that here a gate for genetic research in oyster is wide open. Cross-breeding has been done among several local races of *O. gigas* for the purpose of improving the quality of the Japanese oyster (Imai T. & S. Sakai, 1945, '47 and '49.*), of which the result will be published on a later occasion.

In presenting this paper we wish to express our great thanks to Dr. H. Terao, former director of the Institute of Agricultural Research for the encouragement given to us. Our thanks are also due to the members of Onagawa Fishery Laboratory who took part in the experimental perfomances in past seven years. The authors express their hearty gratitude to Saito Gratitude Foundation in Sendai, Ministry of Education** and Central Experiment Station of Fisheries, Ministry of Agriculture, for the funds providing us tanks and expenses for the experiments.

Onagawa Tanks

Before proceeding to the discussion of the experiment, a brief description of the Onagawa tanks will be given. Three large tanks at Onagawa Fisheries Laboratory were built in 1941 with the fund from Saito Gratitude Foundation in Sendai. They were vertical sided concrete structures of varying capacities with a depth of 1.2 m. They consisted of one tank having a capacity 34,000 litres and two of 19,000 litres. To these culture tanks, one filter tank with the dimension of 1.35 m. × 1.55 m. and a depth of 1.70 m. and one settling tank of a smaller size with the same height were connected. In 1944 eight small square tanks, each of capacity 2,500 litres with a depth

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* Papers were read at the annual meetings of Jap. Soc. of Sci. Fish.

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of 1.2 m., and in 1945 six more tanks of capacity 9,000 litres with a depth of 1.2 m. and a filter and a settling tanks were added. Finally in 1949, the largest tank was divided into four sections of equal capacity by putting in concrete septa. Thus, at present, we have 20 tanks of various capacities comprising of two of a capacity 19,000 litres (A_1 and A_2), four of a capacity 12,000 litres (B_1, B_2, B_3 and B_4), six of a capacity 8,900 litres ($C_1 \ldots . C_6$) and eight of capacity 2,500 litres ($D_1 \ldots . D_8$). It should be noted that these tanks are far smaller than those used at Conway or at the River Yealm already mentioned. In all tanks the concrete is water proofed. Alkaline diffusion from the concrete wall delayed the use of the tanks for the purpose of culture. The effect was particularly severe in the smallest tanks which had larger surface of contact between wall and water, relative to the mass of water contained. It took four years before we could use the smallest tanks for breeding experiment. As the water in the tanks was not renewed after once the larvae were introduced, even a slight diffusion from the wall was fatal to the larvae. Each tank was provided with an overflow outlet with a diameter of 5 cm. at the depth of 10 cm. from the top so that during rainfall the top water of less salinity may flow out of it. This plan worked well to some extent but sometimes we experienced fatal damages due to the heavy rains.

Materials and Methods of Culture

Sea water was pumped from the beach near the laboratory. It was filtered through layers of gravel, sand and charcoal 1.5 m. deep in the filter tank, and then poured into the culture tank through the settling tank. Application of charcoal as a filter seemed to improve the condition of tank water for culture as will be discussed later. The chlorinity of sea water was adjusted to about 17‰ by adding fresh water.

Soon after the tank was filled with water, it was inoculated with a few litres of a dense *Monas* culture which had been prepared previously in flasks. In the breeding experiments prior to 1949, enrichment with cooked soluble starch was also given at the beginning of culture. The amount of starch used differed according to the capacities of tanks. On the average it was 0.5g. to 1.0g. per cubic meter of water at a time. Under such conditions the flagellate, *Monas*, grew rapidly and the density of over 1,000 per cc. was reached in a day or so. Further additions of starch as well as of *Monas* culture were given occasionally depending on the decrease in density of *Monas* in the tank water. Inorganic salts of nitrogen and phosphorus were not added except on rare occasions. Practically in no cases were there indications of a deficiency of nitrogen and phosphorus sources for the

growth of *Monas* in the culture tanks. In sea water, right after it was pumped into the tanks, decomposition of soluble organic matter was promptly accelerated and, on inoculation, a high density of *Monas* ordinarily resulted without any addition of starch. Because of such evidence, it was decided, in 1949, to begin the enrichment of starch when the density of *Monas* dropped as low as 500 per cc.

Oysters used for the experiments were mainly the Japanese common oysters, *Ostrea gigas* Thunberg. Native races from Miyagi, Hokkaido, Hiroshima and Kumamoto were transplanted and cultured, suspended below rafts, in Onagawa Bay. The gonads reached full ripeness in the early part of July and stayed in condition available for artificial fertilization almost through July and August unless they spawned naturally. *O. gigas* is an oviparous oyster and the artificial fertilization could be made easily with ripe gonads. We obtained larvae artificially from eggs and sperm of a set of parent oysters. Fertilization was carried in a Petri-dish having a capacity 500 cc. Fertilized eggs were washed thoroughly by repeated decantations. Then they were transferred to a larger dish with a capacity nearly 2 litres. After four to six hours, depending on temperature, when blastula larvae began floating they were transferred again to glazed earthen jars with the capacity 20 to 40 litres, thus giving enough space for developing larvae. In 20 to 28 hours after fertilization, the larvae developed into the veliger stage and were ready to feed. The density of larvae in jars was measured and the desired numbers were brought into the outdoor tanks for culture.

The tops of tanks were covered with two layers of reed screen to lessen the light intensity. Strong light caused a heavy growth of diatoms and other colord algae and a rise of pH of the tank water to the limit unfavorable for oyster larvae. Cole (1936) reported a successful breeding in waters with pH above 8.5. But in our tank conditions, Japanese oyster never showed good growth in the medium of such high pH value. Moreover, after a heavy growth of diatoms there always occurred a clotting of diatom with which the swimming larvae became entangled. When the tank was covered with double layers of reed screen, the light intensity was reduced as low as 2 to 5 per cent of the direct light, a growth of diatom was inhibited and the culture medium was remained favorable for breeding throughout the period of culture.

One of the difficulties we met in tank breeding was the loss of oyster larvae due to attack by larvae of a mosquito, *Aedes togoi* Theobald, which was abundant in the vicinity. They laid their eggs on the surface of tank water and the hatched mosquito larvae fed on the oyster larvae. Stagnant water in tanks provided them a favorable habitat with ample food. It was

not seldom that we experienced nearly a total loss of oyster larvae in tanks. Only full-grown oyster larvae could escape the attack by predatory mosquito larvae. Several means were considered to keep the tank water free from mosquito larvae. The method we adopted was to use a gabinoid fish, *Chasmichthys dolichognathus* (Hilgendorf) which was common in the beach water around the laboratory and was easily collected in tide pools. By experimental tests it was ascertained that the fish was a good feeder of mosquito larvae but that a fish over 6.5 cm. in length never caught the oyster larvae even of full grown size, for they could pass through the interspace between the gill rakers when swallowed (Imai T. & R. Sato, in preparation). Application of thismethod to the tank culture was quite satisfactory. We set free one or two of the fish in tank whenever the appearance of mosquito larvae was noticed. Usually the mosquito larvae could be eliminated from the tank water in a day or two.

Number of oyster larvae set in tank was 100 or less per litre of water. That is, the maximum population was two million for tanks of the A series, one and a half million for the B series, nine hundred thousand for the C series and a quarter million for the D series.

Gentle stirring was given for few minutes twice a day by means of a hand oar. Temperature, chlorinity, pH, oxygen content and measurement of other chemical and physical conditions were carried at definite intervals during culture. Density of food organisms, size of larvae and their density were also measured regularly. For counting the population density of *Monas*, Lugol-eosine was used for dyeing. Additional enrichment of starch was given when the density of *Monas* dropped less than 500 per cc., occasionally together with a few litre of *Monas* culture.

Results of Breeding Experiments

During the season of 1949, three series of breeding experiments were performed.

Breeding Experiment No. 1.

Materials used were the Hokkaido oyster, a northern type, and the Kumamoto oyster, a southern type of *O. gigas*. The former was the in-bred strain obtained in 1947, while the latter was newly transferred from Kumamoto in the spring of 1949 and were thereafter under culture in Mangokuura farm. They were fertilized on June 30, with the combination shown in Table I. Both oysters were excellent in gonad condition, showed high percentage of fertilization and proceeded with normal development during the early stage.

Table I.
Results of Fertilization in Experiment No. 1.

Oysters		Percentage of Fertilization
Female	Male	
Hokkaido	Hokkaido	94.4 %
Hokkaido	Kumamoto	93.2 %
Kumamoto	Hokkaido	94.0 %
Kumamoto	Kumamoto	93.2 %

On July 1, veliger larvae were transferred to the tanks for culture according to the scheme shown in Table II.

Table II.
Records of Breeding Experiment No. 1. July 1—August 10, 1949.

Culture Number	1	2	3	4	5	6
Tank Number	A—2	C—3	C—4	D - 2	D—6	D—7
Capacity in litre	19,000	8,900	8,900	2,500	2,500	2,500
Parent Oyster Female	Kumamoto	Hokkaido	Hokkaido	Hokkaido	Kumamoto	Kumamoto
Male	Kumamoto	Kumamoto	Kumamoto	Hokkaido	Kumamoto	Hokkaido
Number of Larvae set free in Tank	900,000	950,000	900,000	270,000	200,000	180,000
Tank Water Condition						
Temperature °C	18.7—23.5	17.5—23.5	17.5—23.4	17.7—23.6	17.7—23.8	17.4—23.9
Chlorinity Cl ‰	15.8—16.3	15.8—16.4	15.9—16.3	16.4—16.8	16.0—16.5	15.9—16.8
pH	8.15—8.25	8.15—8.25	8.15—8.25	8.15—8.25	8.15—8.25	8.15—8.20
Oxygen, Percentage of Saturation	86—100	85—99			85—98	
Amount of Starch added, in grams (Date of Enrichment.)	5 (5) 5 (11) 6 (20) 6 (31)	2.5 (5) 3 (13) 3 (19) 5 (31)	2.5 (5) 3 (14) 3 (19)	1 (5) 2 (12) 1 (19)	1 (5) 2 (11) 1 (19)	1 (5) 2 (11) 1 (19)
Monas Population per cc.	100—1,200	100—1,700	100—10,000	400—3,200	100—4,300	200—1,100
Results of Culture. Estimated Number of Grown Larvae	500,000	700,000	600,000	60,000	76,000	87,000
Percentage to Original Population.	55%	74%	67%	22%	38%	48%
Minimum Duration of Laval Stage in Days	30	31	26	32	33	32
Spat Collection	Fairly good	Fairly good	Abundant	Poor	Fairly good	Poor
AverageNumber on One Side of Collector.	5—10	5—10	Over 50	2—3	5—10	2—3

Record of breeding experiment No. 3 is described in Figure 1. As shown in Table II and the Figure, the temperature of the tank water was rather low, particularly during the early half of the culture period. As a consequence, the development of larvae was slow, taking 26 days before the earliest setting of spat was noticed in culture No. 3. It took over 30 days in other tanks. Other environmental conditions such as chlorinity, pH and oxygen content were within the range favorable for larval growth. Density of food organisms, *Monas* sp., seemed to have been kept at the proper level by indicated enrichment of starch.

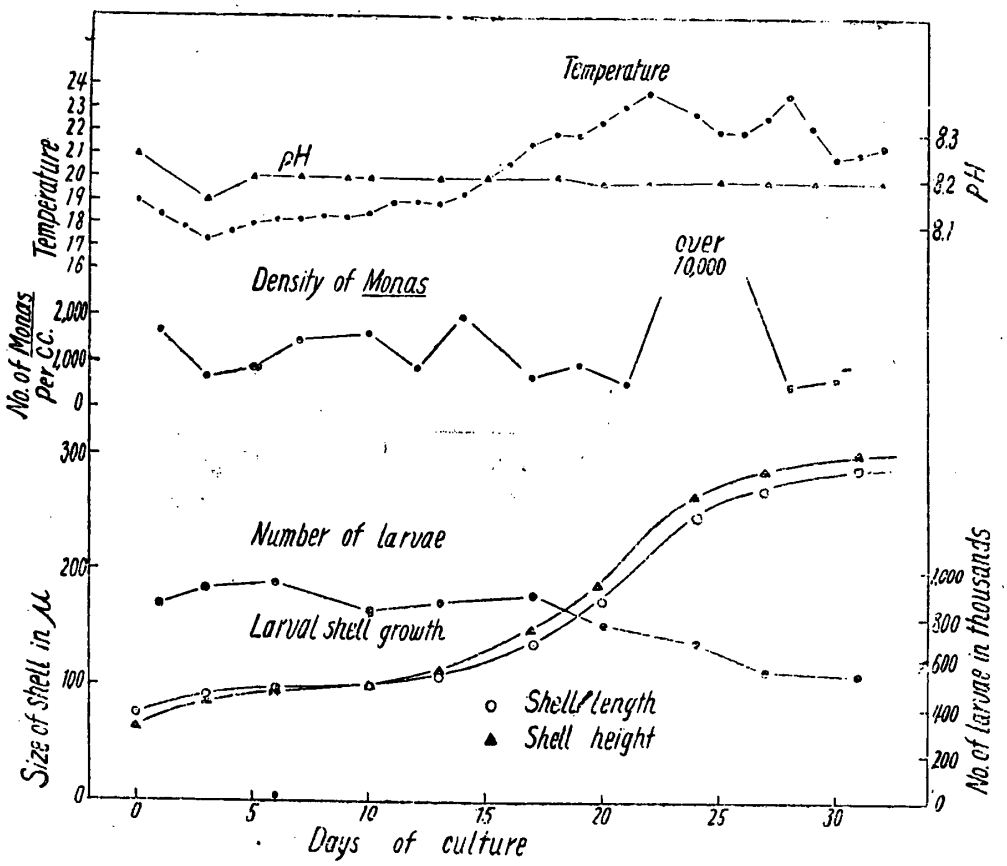

Figure 1. Showing tank-water conditions, density of *Monas*, larval shell growth and larval population in Tank Culture No. 3 of Breeding Experiment No. 1.

Curves of shell growth in Figure 1 is typical of oyster larvae. They began feeding soon after they reached the straight hinge stage. The size of shell at this stage ranged from 60 to 70 μ in height and from 70 to 80 μ in length. Its growth was rather slow. When its length reached around 90 μ, the umbo began to appear and its height gradually became greater than

the length. But the growth of the shell at this phase was very slow, as if it were suspended for a while. Once the larvae passed this stage, the growth went at a good pace until they reached full size. This latter period of growth was characterized by lateral asymmetry and the prominent growth of the umbo. Thus, the growth curve of oyster lavae consisted of two cycles of logistic type. In the culture experiments, high mortality was often experienced at the transitional phase of the two cycles. Larvae which had safely passed this phase grew smoothly without abnormal mortality. Based on these observations, we can affirm that this phase implies the critical period of metamorphosis in the development of oyster larvae.

Table III is an example of the percentage distribution of larval shell length at different stages of culture.

Table III.

Percentage of Population in 0.01 mm. Size Group in Culture No. 3. Size of Sample was 20.

Size	July 1	4	7	11	14	18	21	25	28	Aug. 1
.06	100	—	—	—	—	—	—	—	—	—
.07	—	25	—	—	—	—	—	—	—	—
.08	—	70	35	10	—	—	—	—	—	—
.09	—	5	65	70	25	—	—	—	—	—
.10	—	—	—	20	50	—	—	—	—	—
.11	—	—	—	—	10	5	—	—	—	—
.12	—	—	—	—	15	5	—	—	—	—
.13	—	—	—	—	—	30	—	—	—	—
.14	—	—	—	—	—	40	10	—	—	—
.15	—	—	—	—	—	15	15	—	—	—
.16	—	—	—	—	—	5	15	—	—	—
.17	—	—	—	—	—	—	10	—	—	—
.18	—	—	—	—	—	—	25	5	—	—
.19	—	—	—	—	—	—	15	—	—	—
.20	—	—	—	—	—	—	5	—	5	5
.21	—	—	—	—	—	—	—	10	—	—
.22	—	—	—	—	—	—	5	—	—	5
.23	—	—	—	—	—	—	—	—	15	5
.24	—	—	—	—	—	—	—	15	—	—
.25	—	—	—	—	—	—	—	15	15	5
.26	—	—	—	—	—	—	—	10	5	—
.27	—	—	—	—	—	—	—	25	15	15
.28	—	—	—	—	—	—	—	5	15	5
.29	—	—	—	—	—	—	—	—	20	15
.30	—	—	—	—	—	—	—	10	10	20
.31	—	—	—	—	—	—	—	—	—	15
.32	—	—	—	—	—	—	—	5	—	5
.33	—	—	—	—	—	—	—	—	—	5

This example as well as others which showed a similar type of distribution, emphasize that, though an increased variation in size were observed towards the end of culture period, their development was fairly

normal. Furthermore, taking the mortality during the period of culture into consideration, it can be generally stated that the conditions were fairly suitable in this culture experiment.

The number of larvae surviving at the beginning of the setting, as expressed in the percentage of number started with, was 22% to 74%. Most of the survivors indicated were nearly full grown larvae. It is noticed that the culture in the smallest tanks could not gain the same good result as in the larger ones. Because of the alkaline diffusion, these smallest tanks could safely be used in the experiment in 1948 for the first time after they were built four years previously. Hereafter much better results will be expected from them.

The number of larvae actually settled as spat was difficult to count, because many of them settled on the walls of tanks. The average number of visible spat was counted from those on the collectors as shown in Table II. The best results were obtained in Tank C-4 where nearly 2,000 collectors were covered with 50 to 100 spat on each side. Collection of spat in other tanks was not so good as in this one but fairly good sets were obtained in Tank B-2, C-3 and D-6. In Tanks D-2 and D-7, it was poor with only a very few spat on each collector.

Breeding Experiment No. 2.

Meterials used in this experiment were the Hokkaido race of *O. gigas* and the American oyster, *O. virginica.** The American specimens were transported by air mail to Japan in December, 1948 and have since been under culture in the raft in Onagawa Bay. They were rather thin and showed very poor gonad development. Hokkaido oyster belonged to the same strain used in the previous experiment and their gonad was in excellent condition. As a result, fertilization rate was very low in American oyster as shown in Table IV.

Table IV.
Result of Fertilization in Breeding No. 2.

Combination of Fertilization		Percentage of Fertilization
Female	Male	
Hokkaido	Hokkaido	97 %
O. virginica	*O. virginica*	17 %
O. virginica	Hokkaido	40 %
Hokkaido	*O. virginica*	5 %

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Fertilization was performed on July 19 and veliger larvae were set in tanks on the 20th for culture. The records of culture are summarized in Table V. The larvae obtained by cross fertilization between *O. gigas* and *O. virginica* showed abnormal development in the early veliger stage and none of them survived as far as the stage of umbo development. Therefore their culture records are omitted from the Table.

Table V.

Records of Breeding Experiment No. 2. July 19—Aug. 23.

Culture Number	1	2	3
Tank Number	C—1	C—5	D—3
Capacilty in litre	8,900	8,900	2,500
Parent Oyster Female	Hokkaido	Hokkaido	*O. virginica*
Male	Hokkaido	Hokkaido	*O. virginica*
Number of Larvae, set free in Tank	800,000	800,000	120,000
Tank Water Condition Temperature °C	21.0—23.5	21.1—24.4	21.8—24.3
pH	8.20—8.25	8.20—8.22	8.20—8.25
Oxygen, Percentage of Saturation	100—114	100—115	100—112
Amount of Starch added, in grams (Date of Enrichment)	3 (0) 3 (15) 3 (26)	3 (0) 3 (15)	1 (0) 1 (15)
Monas Population per cc.	100—2,900	300—2,000	100—1,200
Results of Culture Estimated Number of Grown Larvae	430,000	300,000	3,000
Percentage to Original Population	54%	37%	2.5%
Minimum Duration of Larval Stage in Days	31	22	23
Spat Collection	Fairly good	Abundant	Poor
Average Number on One Side of Collector	5—10	50—100	2—3

The temperature condition in this experiment was much better and the larval growth was much faster than in the preceding one. Measurement of larval size revealed that a certain number of larvae reached full size on the 19th to 20th day in culture No. 2 and 3. The duration of larval life of *O. gigas* in nature was estimated as 14 to 18 days at around 25°C. If this estimate was correct, the growth rate under tank conditions was by no means slow as compared to the condition in nature. Heavy settling of larvae was obtained in Tank C-5 while in Tank C-1 only a few spat were

collected, though the survival rate was higher. *O. virginica* in Tank D-3 showed high mortality and poor setting of spat. Such result seemed to be mainly due to the poor quality of gonad as already mentioned.

Breeding Experiment No. 3.

Materials in this experiment were the Hiroshima oyster, and the American oyster. Hiroshima oysters were in excellent condition and the gonads

Table VI.

Result of Fertilization in Breeding No. 3.

Combination of Fertilization		Percentage of Fertilization
Female	Male	
Hiroshima	Hiroshima	93.5%
Hiroshima	*O. virginica*	62.0%
O. virginica	*O. virginica*	89.0%

Table VII.

Record of Breeding Experiment No. 3. Aug. 4—Sept. 3.

Culture Number	1	2
Tank		
Number	A—1	D—5
Capacity in litre	19,000	2,500
Parent Oyster		
Female	Hiroshima	*O. virginica*
Male	Hiroshima	*O. virginica*
Number of Larvae, set free in Tank	1,400,000	200,000
Tank water Condition		
Temperature °C	21.5—24.2	22.1—24.3
Chlorinity, Cl ‰	15.6—18.0	16.8—17.8
pH	8.20—8.27	8.21—8.25
Oxygen, Percentage of Saturation	112—118	96—112
Amount of Starch added, in grams (Date of Enrichment)	5 (9) 5 (18)	1 (7) 2 (12) 2 (22)
Monas population per cc.	100—2,900	300—2,000
Results of Culture		
Estimated Number of Grown Larvae	600,000	5,800
Percentage to Original Population	40%	2.9%
Minimum Duration of Larval Stage in Days	23	28
Spat Collection	Abundant	Poor
Average Number on One Side of Collector	50—100	1—2

were quite ripe, while American oysters were in rather poor condition. Fertilization was performed on Aug. 3 with the combinations below(Table VI).

On Aug. 4, the larvae were set in tanks. The results of breeding are summarized in Table VII. Here again the cross-bred larvae of Hiroshima and American oysters failed to develop beyond the early veliger larvae. Therefore their culture records are omitted in the Table.

Details of culture No. 1 are recorded in Figure 2. Culture condition was favorable except for the heavy rain on Aug. 17 and 18, which was nearly 60 mm. in two days. The damage was evident in the sudden drop of larval density. The rain also caused a sudden increase in the *Monas* density. Despite such a remarkable crisis of environment, heavy setting of spat was obtained. Poor setting of the spat of American oyster was likely due to the poor material involved.

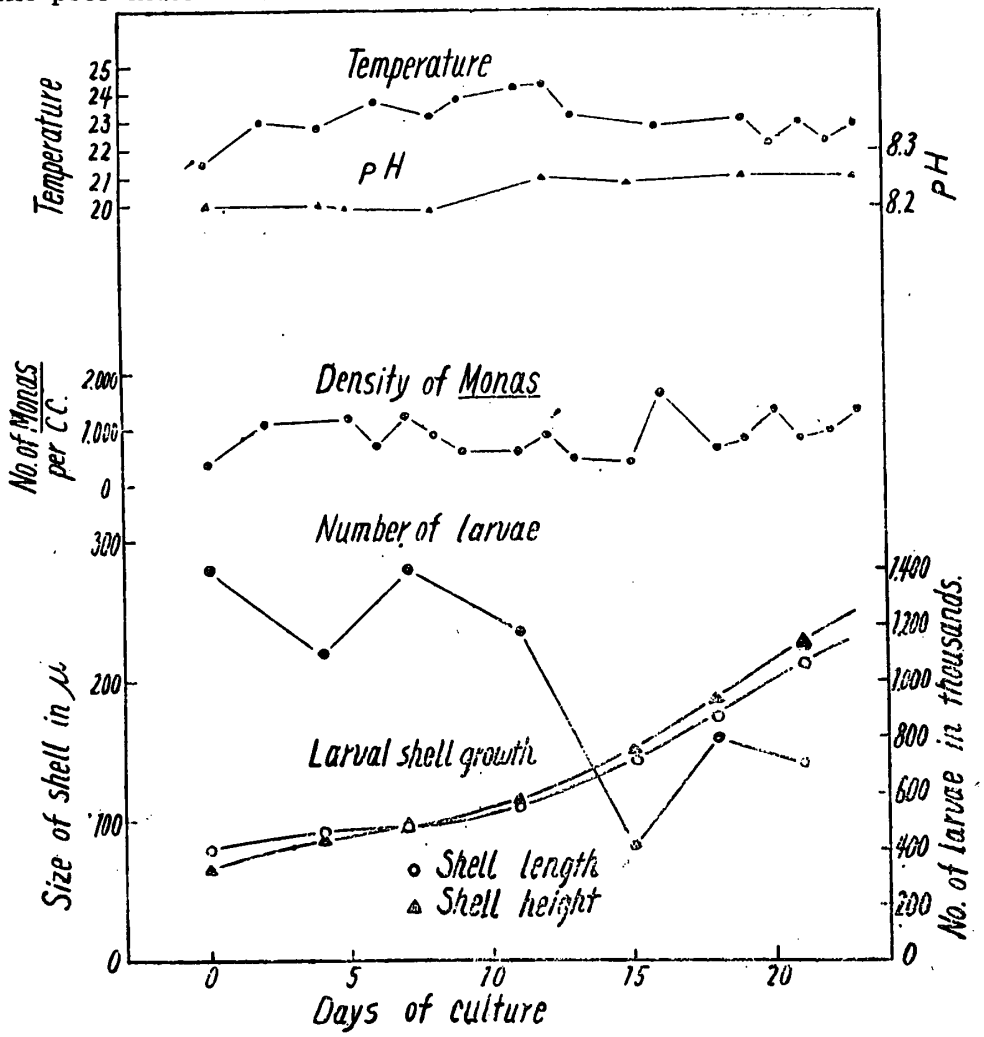


Figure 2. Showing tank-water conditions, density of *Monas*, larval shell growth and larval population in Tank Culture No. 1 of Breeding Experiment No. 3.

Viability of Artificially Bred Spat

Oyster spat which settled on shell collectors were able to continue their growth for a while, even though they were kept in tanks and reached 2 mm. and over in length in a few weeks (Figure 3). This means that young spat could feed on the same food organism as larvae. In our breeding work, however, the collectors, soon after enough setting of spat was obtained, were moved to another small tank with running sea water where they were left until the end of September; they were then transferred to the rafts in Onagawa Bay for culture. The reason for such treatment was to avoid the possible setting of natural spat, because the tank-bred spat were desired for use in genetic work. We could make certain that they remained in better condition by transferring them to the raft soon after their collection (Figure 4).

There is a sceptical view as to the viability of artificially bred spat. Some authorities say that the oyster spat bred artificially, particularly those derived from artificial fertilization, are weak and can not be used for culture purpose. Viability can be proved by the fact that we have been using these tank-bred spat in culture for years, and several strains of oysters have been maintained for generations.

However, in order to compare the viability of tank-bred oysters with that of oysters naturally collected, a test was performed. On Nov. 18, 1947, four groups of spat collected in tanks during the past summer were transferred to the hardening beds in Mangoku-ura. Ten collectors of each group and also ten of natural spat of Mangoku-ura which had been under hardening treatment over a month, were laid on the experimental hardening racks. The racks were set at the level of exposing the oyster spat to air during low tide. Under such conditions the growth of spat was delayed

Table VII.
Mortality during the Hardening Treatment in Oyster Spat.

Kind of Oyster Spat	Number of Spat	Mortality as expressed by Percentage of Deaths			
		Nov. 18	Dec. 8	Feb. 5	March 29
1. Miyagi, Natural Spat	248	0%	4.9%	11.8%	21.7%
2. Miyagi, Tank-bred Spat	171	0%	23.4%	37.5%	38.0%
3. Hiroshima, Tank-bred Spat	218	0%	30.3%	33.9%	44.0%
4. Hiroshima × Hokkaido, Tank-bred Spat	272	0%	10.3%	15.4%	22.4%
5. Hiroshima × Miyagi, Tank-bred Spat	504	0%	12.9%	27.0%	38.5%

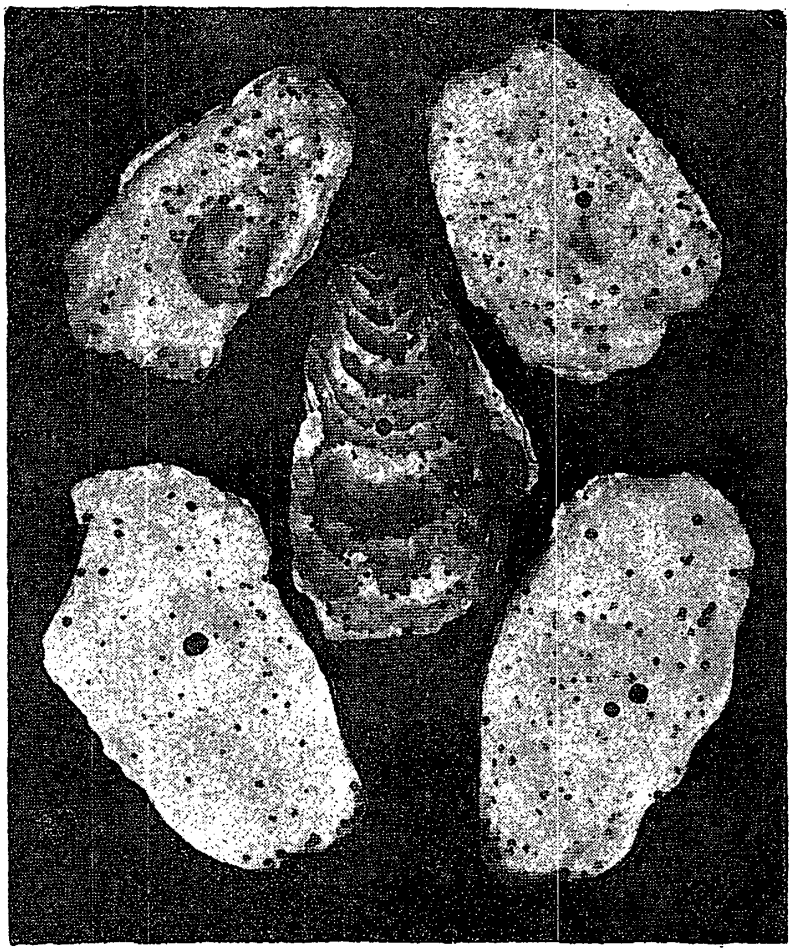
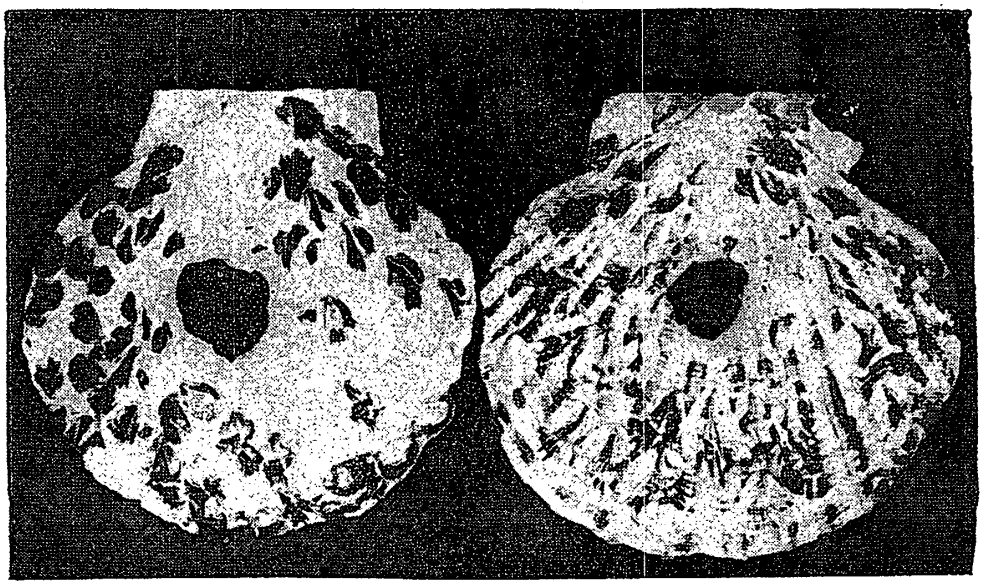


Figure 3. Showing tank-bred spat a few weeks after settled.



. igure 4. Showing tank-bred spat after two months of raft cultu:e.

and hardening of shell occurred. Most of the weak specimens died during this treatment. The results of experiment on hardening treatment are shown in Table VIII.

Death rate of tank-bred spat during four months of hardening treatment was from the order as seen in the natural spat to the order of twice as much as natural spat. That is why the former were transferred directly from raft culture condition and could not help getting rid of high mortality in the very first period of treatment, while the latter had been on the hardening raft over a month and were in better condition at the start of the experiment. Taking such explanation for high mortality in the early period of the treatment into consideration, the viability of artificially bred spat can not be concluded inferior to the natural spat in their availability for practical culture.

Conclusions on the Breeding Experiments of 1949 and Discussion on the Possible Improvement of the Culture Method

In these breeding experiments of 1949, as well as those of preceding years, it was clearly demonstrated that artificial production of oyster spat was possible in tanks by feeding with *Monas*. They proved the possibility of successful breeding in small tanks with a capacity of only 2,000 litres, without renewing the water. The results of setting were by no means uniform, but we have approached a stage wherein we are capable of breeding oyster larvae with uniform results. The principle applied in this culture method was not on the wrong track in the sense that the larvae could be provided with enough supply of food and the environmental condition could be controlled in ways favorable for developing larvae.

The results hitherto presented prove that the method of breeding is satisfactory for such biological work as genetic study. The difficulties in tank breeding reported by many other workers seem to be that they could obtain excellent results in some cases, while in other cases the breedings were a total failure: no uniformity in the results was achieved by any procedure. It is due to the difficulties in controlling the quality and quantity of food organisms, and in managing the condition of the tank water. Unless we are sure what kind of food organisms we are to grow and how to handle them, simple enrichment will not give any uniform results. From such a point of view, and from the result obtained in the reported experiments, we estimate that breeding for successful results are within our reach when both food organism and culture condition are under our control.

Several points, of course, need improvement; they shall be discussed here. The essential part of culture, as already mentioned, is to provide enough food organism and at the same time keep the tank water in favorable condition for the larvae. As food organisms grow in company with the decomposition process of organic matter in sea water, the decomposition process should necessarily be controlled so as to produce ample food organisms within the limit of not spoiling the tank water to a degree harmful to the larvae. Too much enrichment at the beginning of culture caused dense growth of *Monas* but often resulted in high mortality among the larvae. Organic matter initially contained in sea water is decomposed by bacterial activity as a result of confinement in the tank, and served to grow the *Monas* population. Such potential productivity is different in sea waters taken on different occasions. When there is too much organic matters in the sea water, inoculated *Monas* multiplies densely but the tank water often became unfavorable to the larvae.

The use of charcoal for the filtration of sea water was considered helpful to prevent the undesirable, heavy growth of *Monas* which might occur right after the water was drawn in the tank. Thorough analysis of the food cycle together with the chemical condition of tank water is necessary for finding the means of keeping the tank water condition under good management. This analysis is now in progress.

It is very important to adopt measures to get rid of the influence due to rain fall. We frequently experienced fatal damage from heavy rain, though much of the lower salinity water on the top was eliminated through the overflow outlets. Covers should be put on the tanks during rain. We intend to complete the facilities for this procedure during the coming season. Increase in the depth of the tank may also be desirable according to the results shown by Cole on the Conway tanks.

The quality of eggs and sperm used for fertilization unquestionably is an important factor. Therefore, the selection of good material is of fundamental importance. When immature eggs or sperm are used, fertilization may result and veliger larvae may be obtained, but they usually die off sooner or later during the course of culture. For such reason, natural spawning in tank will be preferable to artificial fertilization.

Generally speaking, the oyster larvae are susceptible to a slight change in the quality of the sea water. When once they are exposed to unfavorable conditions, there is no chance that they will recover from the damage they have suffered. Therefore uniform results can only be expected from the careful management of tank conditions.

Summary

1. A new method of breeding oysters in tanks is described. The larvae were fed with the non-colored naked flagellate *Monas* sp., which had been grown in tank water, making use of the decomposition of organic matter.

Population of food *Monas* was controlled by the amount of starch added as an organic enrichment. By reducing the light intensity to less than 5% of the direct light, it was possible to maintain the tank water in condition favorable to larval life through the breeding period.

Loss of larvae due to an attack by mosquito larvae could be avoided by setting free a gabinoid fish, *Chasmichthys dolichognathus.*

2. Records of breeding experiments in the 1949 season revealed that, under the scheme applied in rather small outdoor tanks, fairly high percentage of larvae reached full grown stage and rather uniform settling of spat was obtained.

A stage seems to have been reached wherein we are able to manage the tank-breeding process for the uniform production of oyster spat.

Possible means to improve the method are suggested.

Viability of artificially bred oyster was proved to be fairly close to the natural spat.

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