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# Maturation and Spawning of Flounder (*Paralichthys olivaceus*) under Captive Conditions

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# 넙치 (*Paralichthys olivaceus*)의 종묘생산에 관한 연구
## — 室內飼育 넙치의 成熟과 產卵 —

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## ABSTRACT

The flounder reared in captivity matured and spawned when the water temperature gradually increased to 14~15 ℃ from 12 ℃ during winter and the diurnal photoperiod changed from 10L/14D in winter season to 14L/10D in spring.

The eggs spawned naturally by reared spawners in captivity during the first half of a spawning period were superior in quality to those spawned during the later half. It would be better to use the eggs of the first half for the mass production of the seedlings.

## 요 약

사육 조건하에서 넙치는 사육 수온이 12℃에서 14~15℃로 상승하고 광선 주기가 10L/14D에서 14L/10D로 되는 시기에 성숙하여 산란하였다.

사육 환경하에서 사육된 어미의 산란 기간중 전반기에 산란된 수정란이 후반의 것에 비하여 양질의 알로서 종묘의 대량 생산시에는 전반의 수정란을 활용하는 것이 바람직하다.

## INTRODUCTION

One of the basic prerequisites for the mass production of the flounder seedlings is the stable availability of the viable eggs. Thus, in general, the eggs for mass production of seedlings were obtained from matured spawners from the fishing ground at first step (Midorikawa, 1974 ; Tauti, 1980), occasionally with the help of hypophysation (JFRCA, 1984). Then, the fish caught from wild stocks were reared for maturation and natural spawning in the culture tanks. But the ultimate way of the egg aquisition for mass production is the rearing of the hatchery produced stock in captivity under the controlled conditions for the maturation and the natural spawning, which is under trial in Japan (Kuronuma, 1984 ; JFRCA, 1984).

In this connection, an experiment was carried out to make the flounder mature and spawn in captivity under controlled both water temperature and photoperiod to obtain the viable fertilized eggs for the seedling production.

## MATERIALS AND METHODS

Rearing facility :

The building is 8 m wide and 40 m long and was constructed with 150 mm thick expanded polystyrene sandwiched between steel sheets to insulat the walls and the roof. The floor was also insulated with 100 mm thick expanded polystyrene. Fixed windows were pair glassed and shuttered with insulation panels to cut off the insolation from the outside as necessary.

Inside the building, rearing tanks were built with cement bricks and surfaced with mortar. All the surfaces of the culture tanks and the other chambers and channels were lined with FRPresin. PVC pipes were used in plumbing for water and air.

The culture tanks were equipped with water reuse system. The water reuse system was a modification of the system developed by Kim (1980) for his pilot scale fish production experiment.

The system consists of four circular rearing tanks of 3 m in diameter, one octagonal rearing tank of 8 m in diameter, two circular sedimentation parts and a set of 4 m×8 m filtration chamber (Fig. 1). The water was introduced from 500 $m^3$ reservoir tank to the reservoir (R) in the system, from which the water was pumped to the rearing tanks (RTI-RT5) by five circulation pumps installed in the pumping compartment. Each pump was 300 W and could deliver 15 $m^3$/hr. of water. Two pumps were for rearing tanks RTI-RT4, one for RT5, and other two as back-up. The culture water pumped into the influent ditch (ID) was delivered to the rearing tanks of RT1-RT4 through stand pipes that could control flow rate by adjusting the height.

There were two effluents in each of the rearing tank, one in the center of the tank, and the other on the side 0.3 m high from the tank bottom. At the center of the tank a double stand pipes could remove sunken solid wastes and also could remove floating wastes by removing the outer pipe or sleeve. The waste materials removed through the central drain pipe were received in the sedimentation pit (SPI), where the wastes settled down on the bottom and the supernatant water flowed to the effluent ditch (ED). The settled waste on the bottom of the sedimentation pit was drained out of the system by removing the stand pipe in the middle of the pit from time to time.

Through the main drain pipe which was installed on the tank side 70~90% of the water flowed out directly to the effluent ditch (ED) where the water was introduced to the sedimentation compartments (SCI and SC2).

The octagonal rearing tank received and drained the sea water exactly the same way as the circular tanks. The waste materials in the rearing tank water were drained out through the double stand pipes in the center of the tank to the sedimentation pit and then to the effluent ditch (ED), and the main water was drained out through the drainage pipe on the side of the tank directly to the effluent ditch, and then

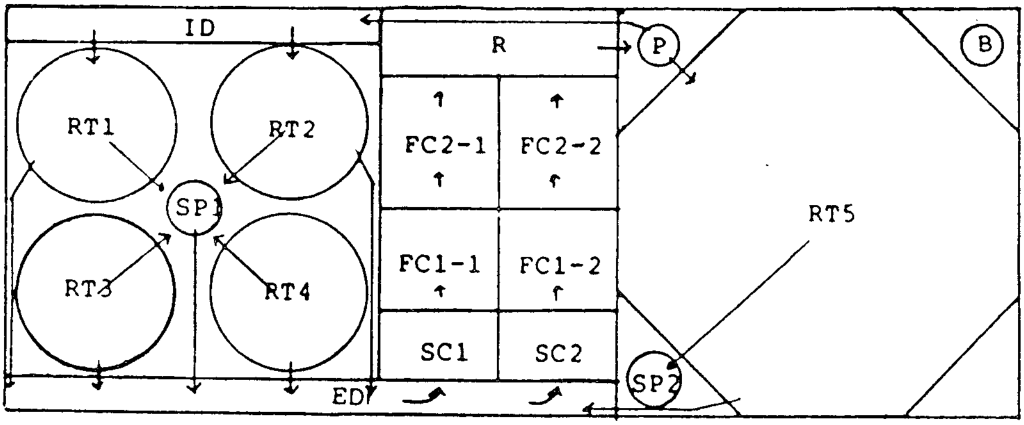


Fig. 1. Plane view of the rearing system.

| | | |
|---|---|---|
| RT1 - RT4 | : | Rearing tanks, ϕ 3 m, 4 $m^3$, circular |
| RT5 | : | Rearing tank, ϕ 8 m, 30 $m^3$, octagonal |
| SP1 | : | Primary sedimentation pit for rearing tanks RT1 - RT4 |
| SP2 | : | Primary sedimentation pit for rearing tank RT5 |
| SC1 - SC2 | : | Secondary sedimentation compartments L1.25×W2 m each |
| FC1 | : | Primary filtration compartments L1.5×W2 m each |
| FC2 | : | Secondary filtration compartments L2×W2 m each |
| ID | : | Influent ditch for RT1 - RT4 |
| ED | : | Effluent ditch for RT1 - RT5 |
| R | : | Reservoir for heat exchanger and culture water circulation pump L1.25×W4 m |
| P | : | Pumping compartment |
| B | : | Boiler compartment |
| → | : | Water flow |

to the sedimentation compartments.

Coarse solid wastes settled down in the sedimentation pits (SP1, SP2) and finer solid wates settled down in the sedimentation compartments (SC1, SC2) as the water flows slowly. The water was then introduced to the biofiltration compartments (FC1-1, FC1-2, FC2-1, FC2-2) in which 400 sheets of corrugated polyethylene skylight plates were installed as biomedia providing a surface area of 1,296 $m^2$. The water passed through the filtraton compartments and flowed into the reservoir from which it was pumped into the culture tanks.

Reuse rate of the water in this experiment was 90 to 97%. Each day about 1,000 $m^3$ of water was recirculated and 30 to 100 $m^3$ of water was supplied in compensation for water which was lost for waste removal and tank cleaning. The amount of the make-up water depended mainly upon the incomming water temperature. To save the heating energy especially during winter when the sea water temperature was around 7 ℃, the quantity of make-up water was kept as little as possible.

Water temperature control :

A boiler of $3.5 \times 10^4$ Kcal/hr. was used to heat the water. The heat was transferred through a heat-exchanger installed in the reservoir. The heat-exchanger was made of titanium pipe $\phi$20 mm and 0.6 mm thick. The total length of the titanium pipe was 36 m. The water in the boiler was heated up to 60 ℃ and circulated by a 40 W pump. A thermostat switch was installed in the reservoir and controlled the circulation pump.

The water temperature of the rearing system was controlled from October 1985 to July 1986 to simulate the temperature mode of the natural habitats of the flounder. The water temperature was maintained above 12 ℃ during the cold season of December 1985 to March 1986.

Illumination :

All the windows were shuttered so that no natural light could interfere the controlled condition of the illumination inside. Each rearing tank was equipped with a set of low fluorescent lamp and 30 W incandescent bulb, which were hung at the center of the tanks 30 cm above the water surface. An automatic illumination controller made a simulation of dawn, daytime and dusk as programmed. At daybreak, an electric timer activated a motor to turn mechanically a rotary transformer up from 0 to 110 V for 1.5 hours. The 30 W incandescent bulb gradually glowed up during the time to make the simulated illumination of the dawn, and vice versa for the dusk. During the daytime from right after the dawn to just before the dusk, the 10 W fluorescent lamp was kept on.

The illumination was controlled to simulate the natural day light at the latitude 35° north (Fig. 2). The luminocity of the light on the water surface of the rearing tanks under the lamps was 300~500 Lux during the time when both of the fluorescent and the incandescent lamps were on. The light regimes were readjusted monthly (Fig. 2).

Stocking and feeding of breeders :

In the beginning of October 1985, the flounder were stocked in the rearing tanks-the stock caught from wild in the octagonal tank (RT5), the stock which were hatched and grown in the Gǒje hatchery in the RT4 and the stock hatched in 1985 in the RT3 (Table 1).

The fish were fed on live fingerlings of grey mullet (*Mugil cephalus*) as forage which were released in the same rearing tanks.

The amount of the live food was amply maintained. As supplementary feed, frozen small mackerel (*Traturus japonicus*) and sand lance (*Ammodytes personatus*) were supplied once a day in the evening. The wastes were eliminated by siphoning next morning. The amount of daily feed was determined depending upon the amount of the supplemental feed remaining in the tank in the next morning.

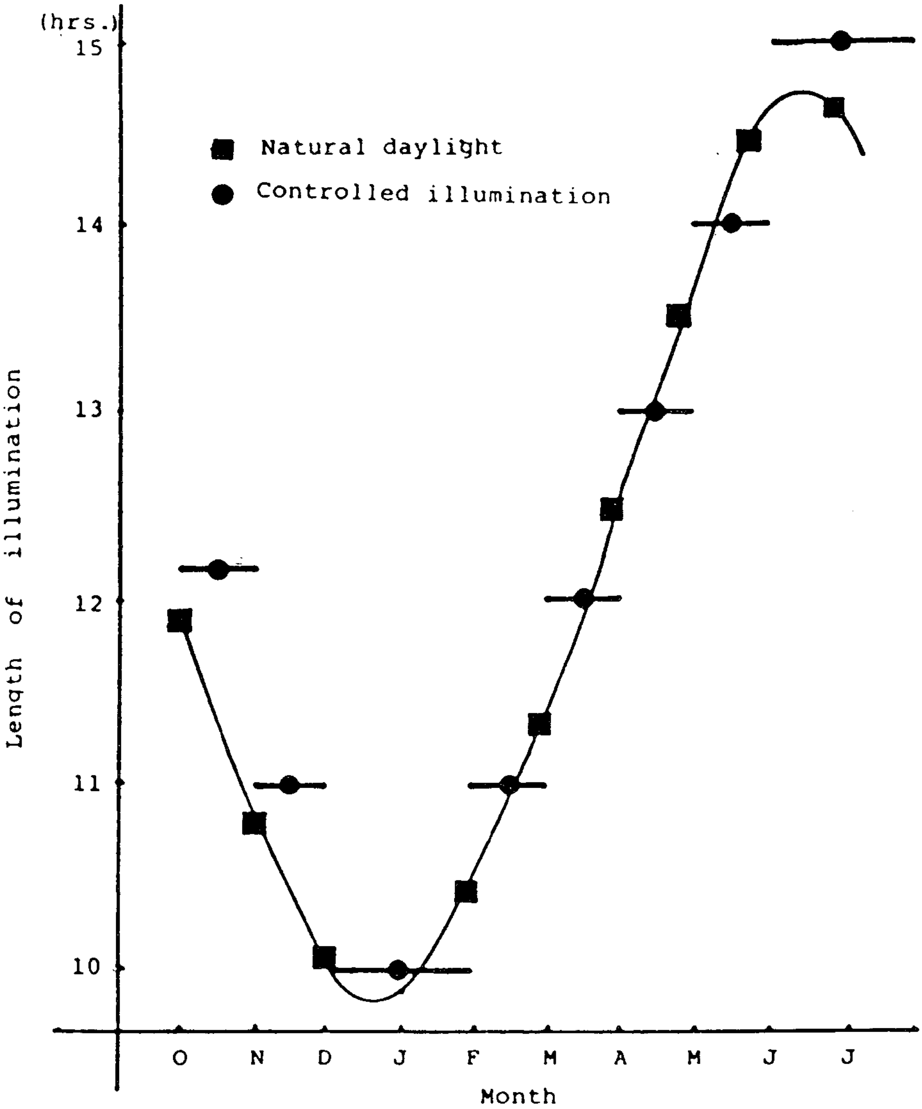


Fig. 2. The natural day length at latitude 35° North and the controlled duration of the controlled illumination in the rearing system from December 1985 to July 1986.

Table 1. The stocking of the flounder for maturation and spawning under controlled conditions

| Tank | No. of fish stocked | Fish origin | Body length (TL, x, mm) | Remarks |
|---|---|---|---|---|
| RT 3 | 20 | Reared | 126± 17 | Hatched in 1985 |
| RT 4 | 12 | 〃 | 274± 63 | Hatched in 1984 |
| RT 5 | 12 | Wild | 631±116 | Caught by angle and stationary net |

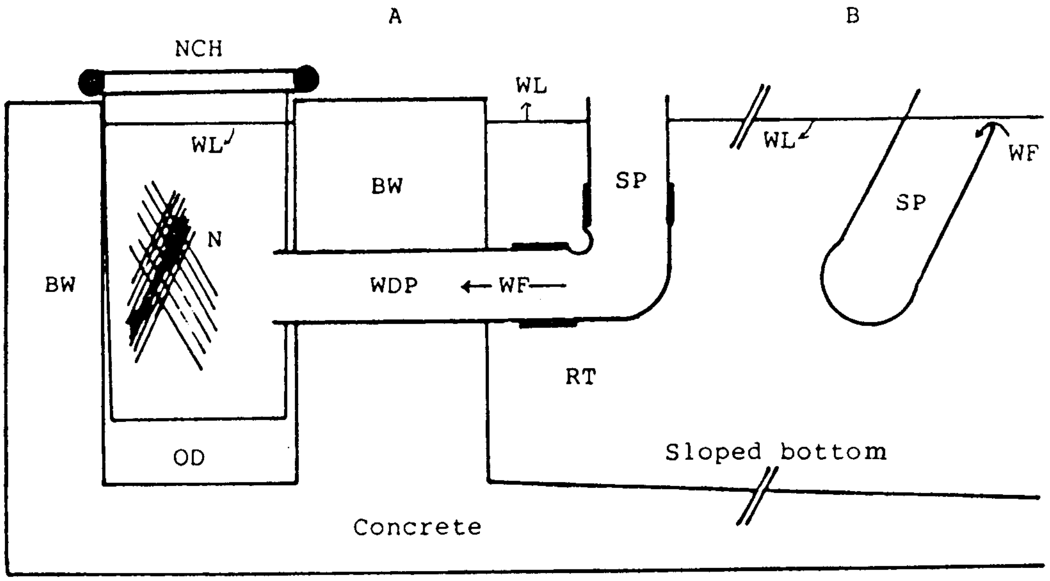


Fig. 3. A schematic diagram of the egg collecting netcage installed in the effluent ditch, A : sectional view, B : front view, WL : water level, NCH : netcage holder, N : net (0.5 mm mesh), ED : effluent ditch, WDP : side drain pipe, RT : rearing tank, SP : stand pipe, WF : water flow with eggs, BW : break wall.

Aeration :

All rearing tanks and sedimentation compartments (SC1, SC2) were equipped with two airstones and aerated continuously for the safety in case of pump breakdown or power failure.

Egg collection :

An egg collector, rectangular box-shaped netcage of 0.5 mm mesh bolting cloth, was installed in the effluent ditch and was connected to the outlet pipe of the rearing tank side. The netcage measured 30 cm wide, 30 cm deep, and 100 cm long. The inner opening of the outlet pipe in the rearing tank was standpiped to drain the surface water with the floating eggs (Fig. 3).

The collected eggs in the netcage were rinsed and transferred into the egg incubators (Fig. 4). One hundred eggs from each spawning were examined under microscope to check the fertility and viability. Dead eggs were collected both in the drain and in the incubator to calculate the hatching rates.

Other factors :

The water temperature and specific gravity were measured every day at 10 A.M.. Special attentions were paid during the experiment not to stress the fish under rearing. No visitors were allowed to enter, and the staffs in charge kept wearing dark colored

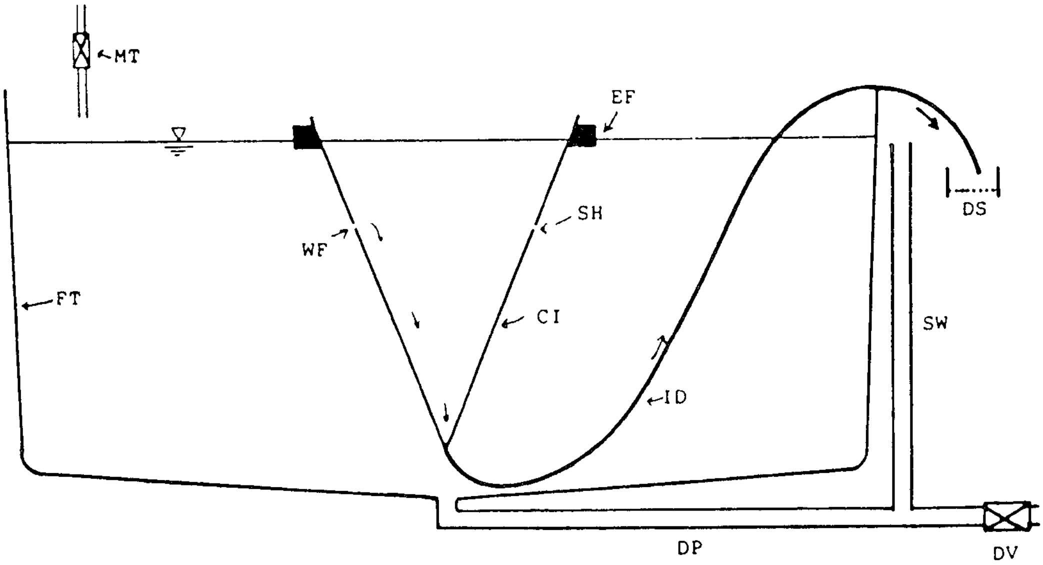


Fig. 4. The schematic diagram of conical incubator installed in circular FRP tank. MT : makeup water tap, FT : FRP tank (ϕ1600×D800 mm), EF : expanded polystyrene float, CI : conical incubator (ϕ500×D600 mm), ID : incubator drain, DP : drain pipe, DV : drain valve, SW : stand-pipe for water level, DS : dead egg collecting seive, SH : small hole (ϕ1.5 mm), WF : water flow.

clothes while they worked on the fish around the rearing tank. Much care was taken not to make any kind of sudden noise other than the continuous noises by circulation pumps and water flows.

## RESULTS

The water temperature in the rearing system during the experiment from October 1985 to July 1986 was plotted in Figure 5. In early October 1985 and late July 1986 the water temperatures were at around 20℃, which was the highest during the experiment and the lowest was 12℃.

The specific gravity of the incoming sea water was summarized in Figure 6. The specific gravity was 1.023 in the beginning of October 1985 and rose up to 1.027 in the beginning of January 1986 and descended down to 1.022 in the beginning of July 1986.

In mid April, three among the 12 hatchery reared fish in tank RT4 and two among the 12 wild origined fish in tank RT5 were checked the swollen abdomen and were expected to spawn soon. The egg collecting devices (Fig. 3) were installed for each tank of RT4 and RT5 at the end of the month.

The first spawning took place in tank RT4 by the hatchery reared fish on May

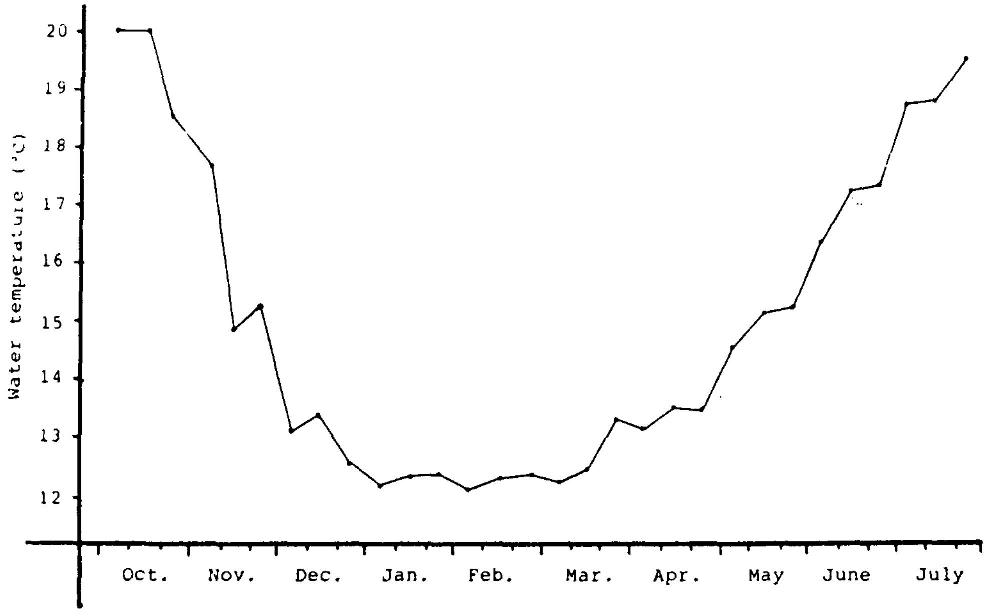


Fig. 5. The sea water temperature controlled during the experiment from October 1985 to July 1986 in the flounder rearing system. The sea water was maintained above 12℃ from December 1985 to March 1986.

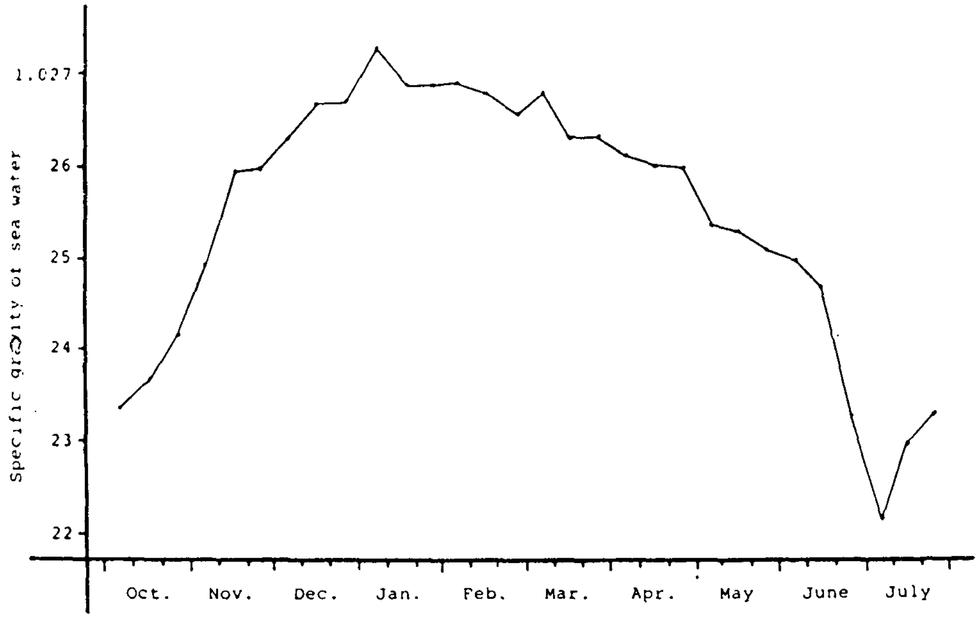


Fig. 6. The specific gravity of the sea water during the experiment from October 1985 to July 1986.

18. The spawning was lasted until July 28. A total of 1,948,000 eggs were spawned (Fig. 7).

The wild origined fish in tank RT5 began to spawn on June 11. Their spawning occurred on July 1. The number of eggs tatalled 274,000 (Fig. 8).

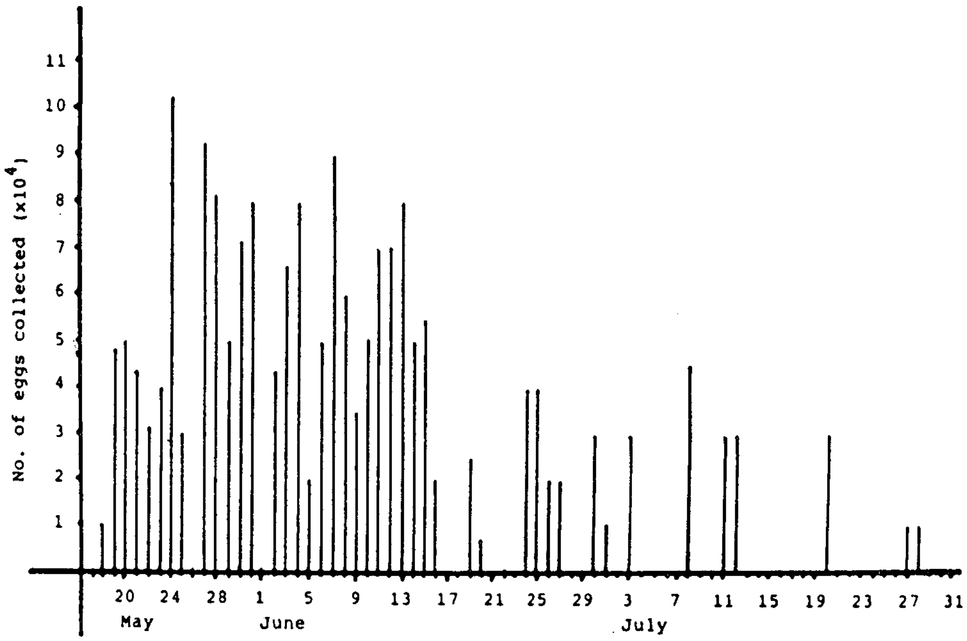


Fig. 7. The number of eggs collected from the tank RT4 spawned by the hatchery reared spawners

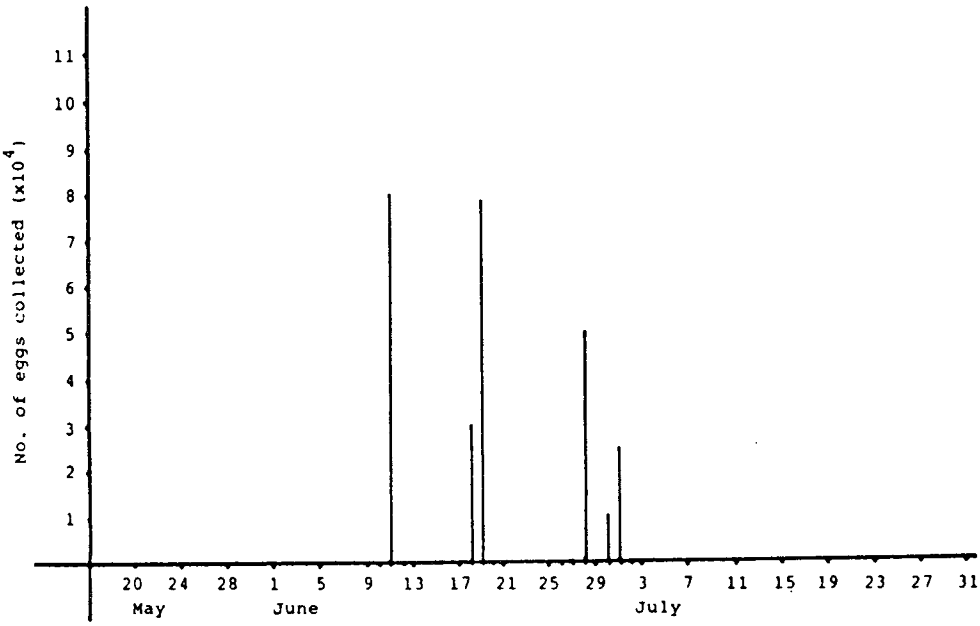


Fig. 8. The number of eggs collected from the tank RT5 spawned by the wild spawners.

The eggs looked good in quality without any noticiable deformities in general. Generally 99～100% of the eggs appeared normal under microscopic observation except for the first and the second batches at the start of spawning and at the resumption of spawning after the intermittent pauses of spawning. The latter case showed a little higher deformity ratio of 4 to 23%. The fertility was always 100% of the normal eggs. The hatching rates fluctuated from batch to batch. Some batches showed total mortality in their morula to gastrula stages. The hatching rates were 72 to 97% except for the batches of total mortality.

## DISCUSSIONS AND CONCLUSION

Spawners :

The hatchery reared spawners, which were produced from the wild parents through egg stripping and fertilization procedures in May 1984, i.e., exactly 2 years old, deposited viable eggs. The first spawning of the 3 years old flounder was mentioned by Kitani (personal communication). But full 2 year period seems to be the minimum age for maturation and spawning in cativity.

The number of eggs deposited by three of the hatchery reared fish was about $2\times10^6$ in a spawning season of 76 days. Hiramoto (1979) used four 5 years old fish (BL of 500～727 mm) and obtained $4.2\sim10^6$ eggs per fish during a 106 day spawning season, thus amounting 6 times of the number of the present experiment. The duration of spawning season of the present 2 years old fish was about 72% of that of the elder ones. But the actual egg-layings occurred only on 43 days of the 76 day spawning season (57%) in the younger fish and for 78 of the 106 days (74%) in the elder ones. Even though the minimum age of the flounder for the spawning in captivity is full 2 years, it was found that the number of eggs, the duration of the spawning season and the actual egg-laying days were all inferior to the elder spawners. For mass production practice of flounder seedlings it seems to be practical to keep the fish no less than full 3 years old as a brood stock.

The wild origined stock in the rearisng tank RT5 deposited 274,000 eggs during 21 days of spawning season from June 11 to July 1. The actual egg layings occurred only on 6 days in the period. The number of eggs, the duration of spawning period, and the actual days of spawning were almost negligible in comparison with the results obtained by the hatchery origined spawners. Hiramoto (1979) reared his flounder for one year before the spawning in tank. He started with 17 fish in March 1976. Among them 4 females and 2 males survived and engaged in the spawning in March 1977. The fish in the present experiment were introduced too late. It can be suggested that the brood stock be reared in captivity at least more than one year before spawning.

Photoperiod :

The photoperiod regime by controlled illumination and the spawning season were illustrated in Figure 9. During the months of December and January the fish received

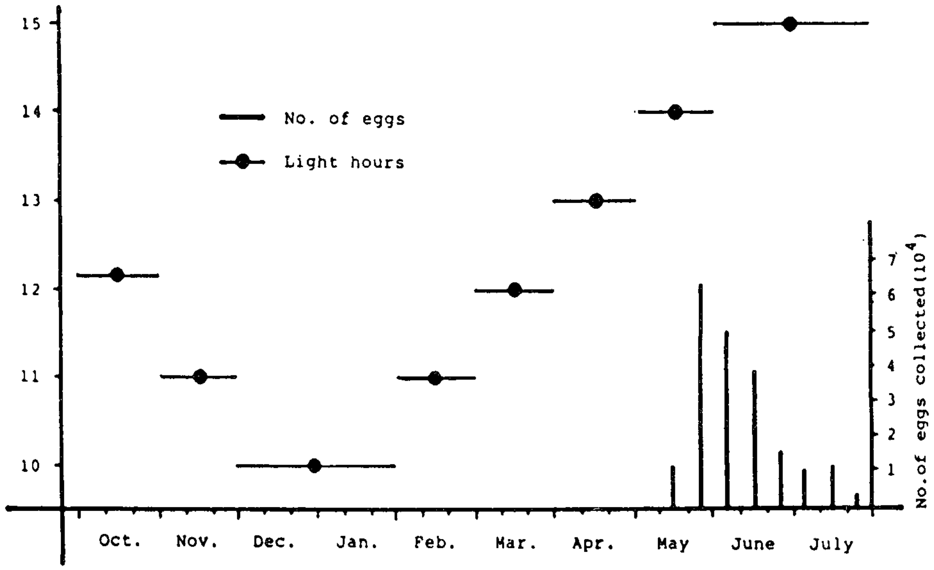


Fig. 9. The hours of controlled illumination for controlling the photoperiod on the flounder maturation in the rearing system during the experiment from October 1985 to July 1986 and the number of eggs during the spawning season.

10 hours of light, and the lighting hours were lengthened one hour a month. In April light regime was 13L/11D. The three fish among the hatchery origined fish and two of the wild origined fish were observed to be gravid. In May, when the light length attained 14L/10D, the brood stock began to spawn. The relation between the photoperiod regime and the maturation of the brood stock was not clearly demonstrated in this experiment. A series of detailed experiments would be required to figure out the photoperiod effects on the flounder maturation and spawning. An increasing mode of light length, or a certain absolute length of light, or both may be effective on the maturation and spawning.

Water temperature :

The water temperature during the experiment including the spawning period was depicted in Figure 13. The temperature ranged 12~20℃ from October 1985 to July 1986. Hiramoto (1979) supplied natural sea water of 10~28℃ for his brood stock and the Study Group on Seedling Production of the Northern Part of the Japan Sea (1984) suggested the rearing water of 8~25℃. In April when the water temperature was between 13~14℃, the gravid females were observed, and the egg deposition took place in mid May when the water temperature was 14.5℃ (Fig. 10). The lowest temperature of 12℃ during the winter months of present experiment followed by the rising temperature seemed to fall in with the safe range for the brood stock rearing. Even higher temperature during the winter season would be worthwhile to be tried for the out-of-season spawning of the seedlings.

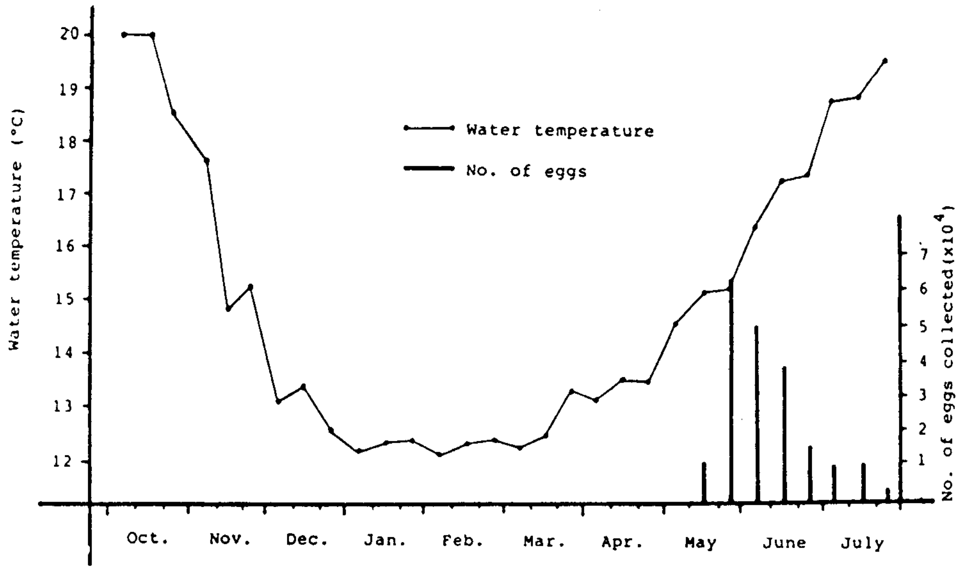


Fig. 10. The water temperature of the flounder rearing system from October 1985 to July 1986 and the number of eggs collected during the spawning season.

The daily fluctuation of water temperature may affect the spawning (Fig. 11). On May 18, the spawning started when the water temperature attained 14.5 ℃ which was the same temperature reported by Hiramoto (1979). The pauses of spawning on May 25, on June 17 and 18, and on July 13 seemed to be strongly related with the temperature drop. The fish resumed spawning when temperature rises. The fluctuation of about 1 ℃ during the spawning period seemed to affect the spawning as stimuli. The water temperature during the spawning season in this experiment was not controlled to allow the temperature fluctuation (Fig. 11). There could be a possibility to make the fish spawn continuously without pause during the spawning period by controlling the water temperature not to fluctuate.

Specific gravity :

The specific gravity during the experiment as well as the spawning season and the number of eggs deposited is shown in Figure 12. The flounder migrate to the coastal areas from their deeper habitats for spawning (Kuronuma, 1984) when the specific gravity is lowered by the spring precipitation. Even though the change of the specific gravity is not directly related with the maturation and spawning of the fish, it is empirically proven that the flounder can spawn even in the sea water of specific gravity of 1.022 to 1.025.

Egg deformity and hatching rates :

Among the eggs deposited during the spawning period, some eggs deformed in their membrane and egg york under microscopic examination. The percentage of normal

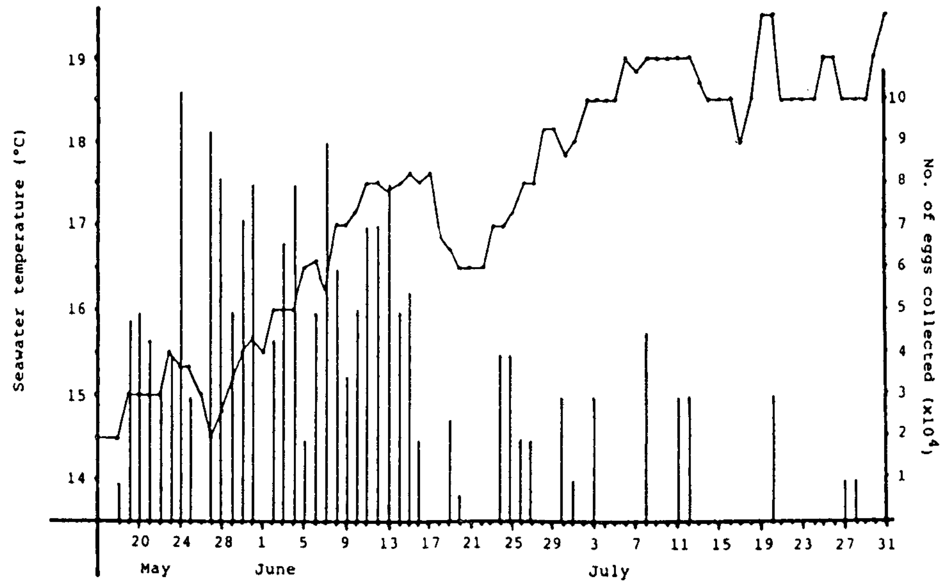


Fig. 11. The daily water temperature and the number of the eggs during the spawning period from the 18th of May to the 28th of July 1986.

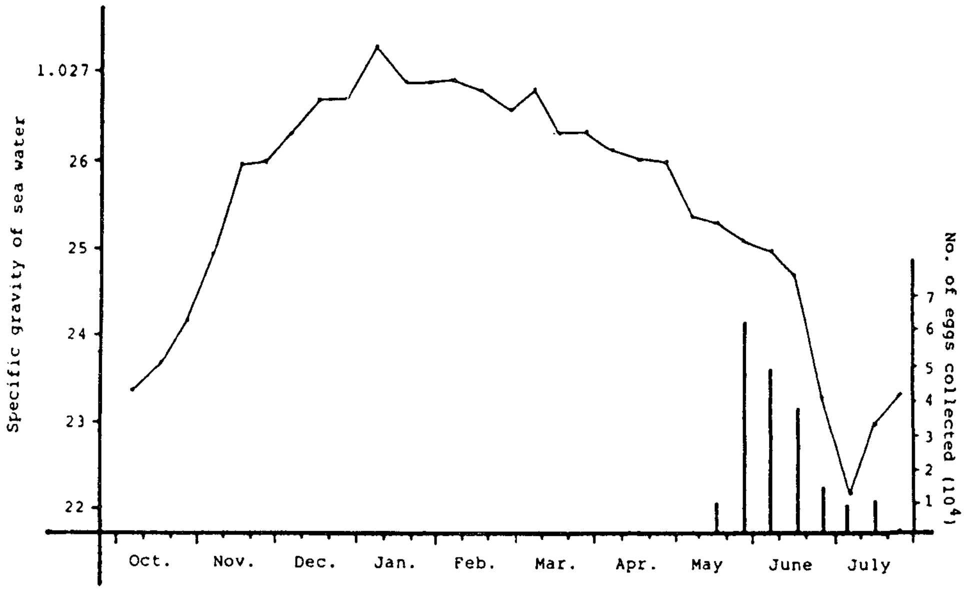


Fig. 12. The specific gravity of the rearing water for the flounder from October 1985 to July 1986 and the number of eggs collected during the spawning season.

eggs are shown in Figure 13. More deformed eggs were found at the beginning and the later half of the spawning period, ranging 4 to 23% of the deposited eggs. The eggs spawned during the first half of the spawning period were more viable and also the hatching rates were higher (Fig. 14) than those during the later half period. For the mass production of the flounder seedlings it can be recommended that the eggs deposited during the first half of spawning period be used for practical production.

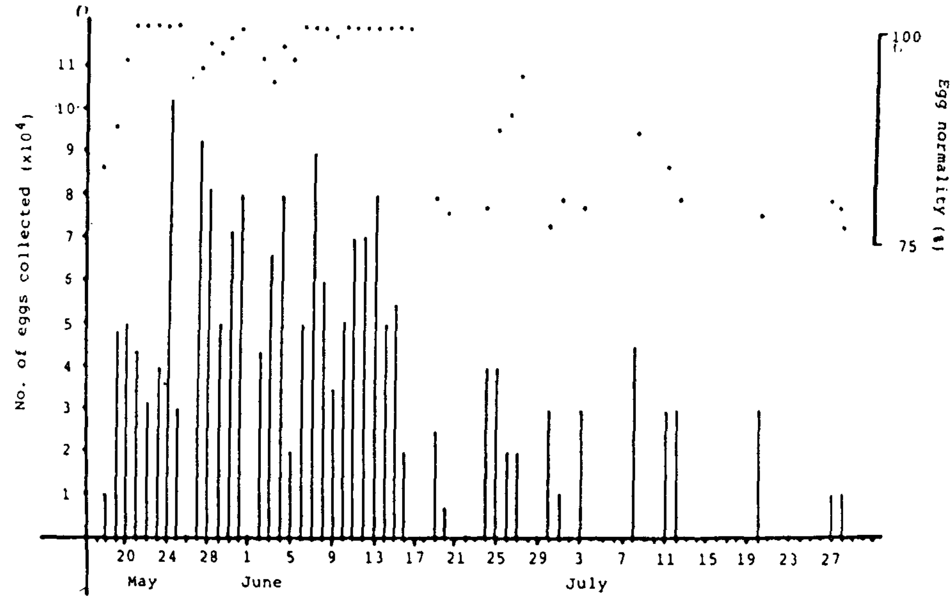


Fig. 13. The number of eggs deposited during the spawning period from the 18th of May to the 28th of July 1986 and the normality ratios (•) of the eggs.

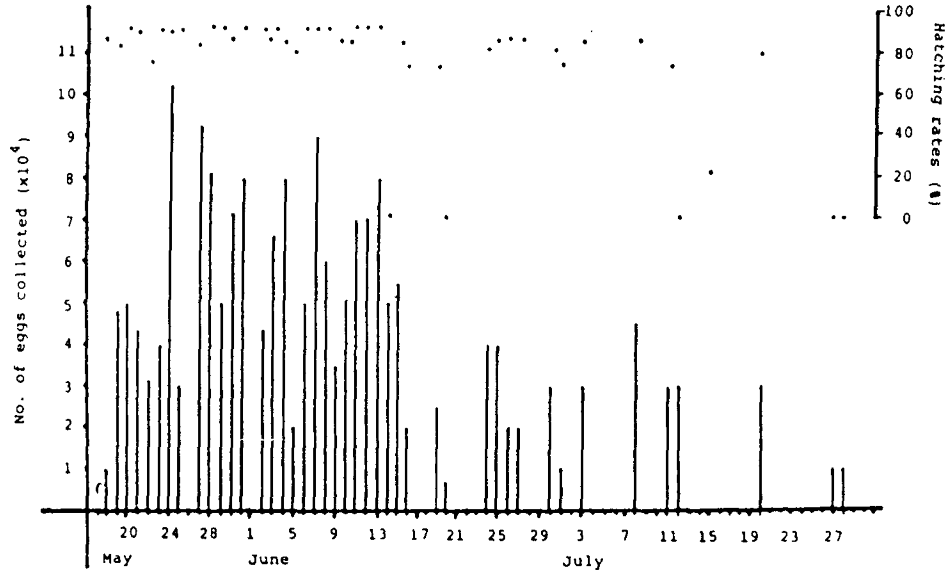


Fig. 14. The number of eggs deposited during the spawning period from the 18th of May to the 28th of July 1986 and the hatching rates (•) of the eggs.

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