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Review

Recent Progress of Research on Larvae Production of Japanese eel, *Anguilla japonica*

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Abstract: The Japanese eel, *Anguilla japonica*, is one of the most important species for freshwater cultivation in Japan due to its popularity as a food fish. However, number of eel fry (glass eel) for the cultivation, which were captured from the wild field, has gradually decreased during the past 25 years, resulting that the price of the fry has risen sharply. Therefore, developments of techniques for artificial breeding of the eel have been desired eagerly. In this paper, we will describe recent researches on artificial induction of maturation for male and female eels and rearing techniques for eel larvae that have been mainly studied in our institute.

Key words: Japanese eel; Artificial breeding; Induced maturation; Larval rearing

Introduction

The total yearly consumption of eel reaches more than 100,000 tons in Japan. Within the total amount of consumption, 70,000 to 80,000 tons of eel were imported from China, Taiwan and other countries. The aquacultural production of Japanese eel, *Anguilla japonica*, in Japan is about 22,000 tons a year in recent years. Eel fry for aquaculture are now totally dependent on the natural glass eel captured in estuaries. However, the amount of glass eel has gradually decreased over the past 25 years. In contrast, the price of the glass eel has risen high and exceeded on million yen per kilogram in 1997. Therefore, developments of techniques for artificial breeding of the eel have been desired eagerly.

In Japan, techniques for artificial breeding of the eel have been intensively studied since 1960s. In 1973, Dr. Kiichiro Yamamoto and his colleagues, Hokkaido University, succeeded in the production of eel larvae (so-called preleptocephalus) from artificially induced mature male and female eels first in the world[1,2]. However, although other workers have succeeded in obtaining fertilized eggs since then, no one has been able to produce glass eel. This may be caused by the following primary problems. Firstly, the percentages of ovulated female are very low, and even if ovulated eggs are obtained, these eggs show low fertility and hatchability[3,4]. Secondly, as the results of unstable production of good quality eggs, an attempt has not been made on development of rearing techniques of eel larvae. To obtain good quality eggs constantly and produce eel fry (glass eel) as a final goal, we have conducted the experiments on the following subjects. 1) Development of techniques for inducing maturation and ovulation in the female eel. 2) Development of techniques for obtaining good quality spermatozoa from male eel. 3) Development of rearing techniques for eel larvae.

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Induction of oocyte maturation and ovulation

Cultivated female eel or silver eel that migrates down rivers were used as experiment fish for artificial maturation. The gonadosomatic indices (GSI) of female silver eel are 1-2% and they have ovaries containing oocytes at the oil stage or oocytes at the primary yolk globule stage[5-7]. Cultivated females also have ovaries containing oocytes at the oil stage. Our recent research[8] showed that GSI (1.4-1.6%) and oocyte diameter (168-195μm) increased when the female cultivated eel (30 month old) kept in seawater for 3 months. Some of them had oocytes at the primary yolk globule stage in their ovaries. These observations are similar to those of the silver forms of wild female Japanese eels, showing that they have oocytes at the oil drop stage or at the primary yolk stage about 200μm in diameter. These results suggest that females reared in seawater for 3 months induce vitellogenesis similar to natural migrating silver eel. However, they never mature and ovulate under the normal cultivated condition. Therefore, techniques for artificially inducing maturation of the eel have to be developed to obtain matured and ovulated eggs.

As Yamamoto *et al.*[9] reported, weekly injection of salmon pituitary extracts (SPE) to female eel at a concentration of 20 mg/fish induced vitellogenesis and oocytes reached the migratory nucleus stage. They used the migrating silver eels that have oocytes at the primary yolk globule stage. This is an advantage for inducing sexual maturation in the silver eel, since the number of injections of SPE which accompanies a lot of labor can be reduced. Our recent study[8] showed that the mean number of injections of SPE until oocytes attain the migratory nucleus stage was significantly less in the eels kept in seawater for 3 months than in the eels kept in seawater for 1 week or 1 month. The number of injections of SPE (8.5±0.2) for inducing maturation in the eel kept in seawater for 3 months was comparable to those in silver forms of female administrated with SPE (about 35 mg/fish/week)[9]. These data indicate that a prolonged rearing period in seawater reduce the period needed until the oocyte attain the migratory nucleus stage. However, although most female eel complete vitellogenesis after repeated injections of SPE, their oocytes do not undergo final maturation and become over-ripe with ooplasmic degeneration in further injections of SPE[3]. Therefore, the development of a technique to induce final maturation of oocytes and ovulation are necessary in the Japanese eel.

Yamauchi and Yamamoto[4] reported that *in vitro* administration of 17α, 20β-dihydroxy-4pregnen-3-one (DHP) into the incubation medium and *in vivo* injection of DHP induced final maturation of oocytes and ovulation in the Japanese eel. Our *in vitro* studies[10] demonstrated that oocytes at the migratory nucleus stage ranging from 700 to 800μm in diameter underwent GVBD in response to DHP and oocytes over 800μm in diameter became more sensitive to the steroid. These results suggest that oocytes acquired the ability to respond to the maturation-inducing steroid at the migratory nucleus stage and oocyte diameter can be utilized as a reliable indicator of the induced maturation and ovulation by the injection of the steroid.

Actually, injection of DHP (2μg/g BW) induced final maturation of oocyte and ovulation in Japanese eel[11] (Fig. 1). Females, showing body weight indices (body weight/initial body weight ×100) of over 110% with oocytes at the migratory nucleus stage (800-900μm in diameter), received an injection of SPE as a priming dose followed 24h later by DHP. Most of females ovulated around the time of 17-20 h after the final injection, suggesting that ovulation induced by the DHP injection occurs in a short and well-defined time within a period of day. Even if the time of DHP injection was changed from 9:00 to 18:00, a majority of the fish ovulated 18 h after DHP injection[12,13]. These data indicate that the time of ovulation depends on the time elapsed after the DHP injection, not on a circadian rhythm. Thus, a shift in the time of DHP injection can change the time of ovulation, indicating the possibility that ovulation can be induced at a desired time in accordance with the time of DHP injection.

Induction testicular maturation and artificial fertilization

Recently, testicular maturation of the male eel has been induced in cultivated males about 200-350 g in body weight by injection of human chorionic gonadotropin (HCG)[14] (Fig. 1). Repeated injection of HCG at a concentration of 1 IU/g body weight induces testicular maturation. After 6 times of injection, most fish spermiated small volume of milt and after then milt volume increased and became stable after 11th injection (1-2 g). Sperm motility (the percent motility of spermatozoa at 15 sec after dilution with 450 mM NaCl solution) also increased and became stable after 9th injection. However, large individual differences in the sperm motility (from 10 to 90%) were observed in male induced to mature by artificial means. And also their sperm motility changes during each weekly injection of HCG. Sperm motility increased sharply one day after injection and gradually decreased on 2 or 3 days after injection and retained at low level. These periodic changes in percent motility may affect the fertilization rates. Thus, in order to obtain sufficient volume of stable motility of spermatozoa, the following dilution method was developed.

We found that sperm motility increase in association with the increase of seminal plasma by the injection of HCG, indicating that seminal plasma may affect the sperm motility in the eel[12]. An intimate relationship was found between sperm motility, milt pH and potassium concentration in the seminal plasma. Percent motility of spermatozoa in the milt were high when both pH ($>$8.05) and potassium concentration of seminal plasma ($>$20.85 mM) were higher enough[15]. These results indicate that the increase of potassium ions and decrease of protons in the seminal plasma stimulate the acquisition of sperm motility in the Japanese eel.

These findings lead the idea that media mimic seminal plasma may be useful as a solution of increase of sperm motility[16]. An artificial seminal plasma (ASP) which is mimicked the ionic constituents (149.3 mM NaCl, 15.2 mM KCl, 1.3 mM $CaCl_2$, 1.6 mM $MgCl_2$, 20 mM $NaHCO_3$ and buffered at pH 8.1 with 20mM TAPS-NaOH) was produced. After spermatozoa were diluted and stored 60 min with ASP, sperm motility was assessed in 450 mM NaCl. Incubation of spermatozoa with ASP increased the percent motility when compared to sperma-

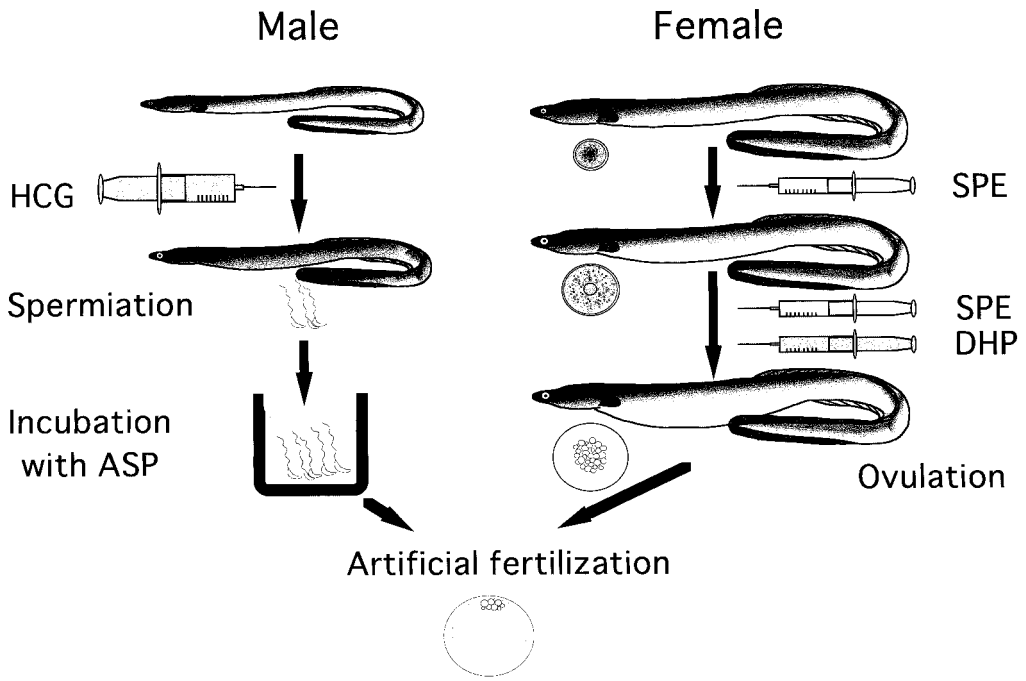

Fig. 1. A summary of the artificial induction method of maturation and fertilization in the Japanese eel. HCG, human chorionic gonadotropin; ASP, artificial seminal plasma; SPE, salmon pituitary extract; DHP, 17α,20β-dihydroxy-4-pregnen-3-one.

tozoa treated with isotonic solution adjusted with NaCl and Mannitol. Moreover, if the potassium ion or bicarbonate ion was omitted from ASP, percent motility decreased sharply to the low levels.

For artificial fertilization of Japanese eel[12], diluted spermatozoa with the ASP were stocked in refrigerator one day before fertilization and artificial fertilization are performed using ovulated eggs which were obtained from the female injected with salmon pituitary extract and DHP as mentioned previously. When ovulated eggs were periodically stripped at 3h intervals, both fertility and hatchability of each fish showed a marked decrease by 6 or 9h after the ovulation[11]. This indicates that delay of fertilization after ovulation causes a decrease of fertility and hatchability, suggesting that artificial fertilization must be carried out immediately after ovulation in order to obtain good quality eggs. However, we found that even if artificial fertilization was performed just after ovulation, the fertility and hatchability of ovulated female varied[13]. Although the reason was not clear, females ovulating 15 h after a DHP injection have better quality eggs (fertility 62% and hatchability 54%) than females that ovulated at 18 (28% and 18%) or 21h (3.2% and 0.8%) after the injection[13]. If the time needed for final maturation of oocyte and ovulation was shortened in all females used for artificial maturation, the quality of eggs may be improved. Using these newly developed techniques for induction of maturation, we are able to obtain relatively large amount of fertilized eggs constantly.

Rearing techniques for eel larvae

Fertilized eggs began to hatch about 40 hs after fertilization at a water temperature ranging from 22 to 23℃, larvae are 3.7 mm long and have oil droplet and yolk. By 7th day after hatch, several marked changes are observed, such as pigmentation of eye[17], synthesis of digestive enzymes in the pancreas[18], completion of yolk protein. Observations of developmental changes suggest that larvae can take feeds from the outside and digest them[19]. However, the preleptocephalus larvae could not survive beyond the depletion of their yolk and oil droplet, since suitable larval feeds were not identified. We first reported that preleptocephalus larvae ingested rotifer, the most common initial feed used in the production of marine fish fry[17]. However, even the larvae that ingest rotifer did not grow much larger and their survival period did not extend. Thus, to identify suitable larval feed, the following feed items were tired to feed on eel larvae; zoo planktons, micro-formulated diets for marine fish larvae and for crustacean, fish egg, cuttlefish, shrimp, jellyfish, gonad of mussel and so on. Among them, finally, we found that eel larvae actively ingest a slurry-type diet made from shark egg powder. The freeze-dried shark egg powder (Aquaran, BASF Japan) is supplied as a feed for improving the nutritional value of food organisms for the culture of marine fish larvae. Eel larvae actively ingest the slurry diet on the bottom of the round acrylic resin tanks. Slurry diet was fed five times a day, every 2 hrs from 9 to 17 o'clock. And the larvae were transferred from the rearing tank to new clean tank after the last feed of the day. Eel larvae grew up to about 8.12 mm in total length and the survival rate was 56% on 18th day with this feed[*2].

This is the first finding that eel larvae survive and grow by the artificial feeding. However, they died around 24th. This means that the more adequate or sufficient feed is needed for continuing their growth and extending their life. We newly mixed the following food items to shark egg powder, such as soybean peptide (Hinute-R, Fuji Oil, Japan), which is supplied as a food for human, and vitamin and mineral mixture for fish[20]. These items were mixed with extract of krill and made a slurry diet. The eel larvae continued to survive and grow up linearly up to 50 days after hatching, their total length attained about 16 mm on the day. On 100 days, they raised to 22 mm in total length and body proportions of the reared specimens overlaps with those of wild leptpocephali (Fig. 2). These

[*2] Abstract for the Meeting of the Japanese Society of Fisheries Science, pp.122, April 2000.

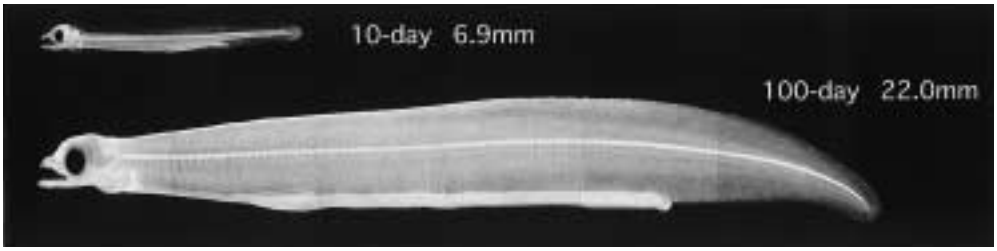

Fig. 2. Captive-bred preleptocephalus (10 days after hatching) and leptocephalus larvae (100 days after hatching).

indicate that we succeeded for the first time to rear the eel larvae to leptocephalus.

However, the survival rates were about 4% on 50 days and decreased 1% by 100 days after hatching. Moreover, the growth rate was nearly half that of wild Japanese eel leptocephali at the same days after hatching[20]. This may be partly due to an incomplete diet, limited feeding period in a day and other environmental factors different from their natural habitat. Further studies should be focused on the nutritional adequacy of diets, lighting and feeding regimes, and optimum temperature to produce the glass eel from the in the cultured leptocephali through metamorphosis.

References

1) Yamamoto, K. and K. Yamauchi (1974): Sexual maturation of Japanese eel and production of eel larvae in the aqualium. Nature, **251**, 220-222.

2) Yamauchi, K., M. Nakamura, H. Takahashi, and K. Takano (1976): Cultivation of larvae of Japanese eel. Nature, **263**, 412.

3) Sugimoto, Y., Y. Takeuchi, K. Yamauchi, and H. Takahashi (1976): Induced maturation of female Japanese eels (*Anguilla japonica*) by administration of salmon pituitaries, with notes on changes of oil droplets in eggs of matured eel. Bull Fac. Fish. Hokkaido Univ., **27**, 107-120.

4) Yamauchi, K. and K. Yamamoto (1982): Experiments on artificial maturation and fertilization of the Japanese eel (*Angulla japonica*). In "Reproductive Physiology of Fish" (ed. By C. J. Richter and G. J. Th. Goos), Pudoc Press, Wargeningen, pp. 185-189.

5) Satoh, H., N. Nakamura, and T. Hibiya (1962): Studies on the sexual matuation of the eel. I. On the sex differentiation and the maturating process of the gonads. Nippon Suisan Gakkaishi, **28**, 579-584.

6) Sakai, K., M. Nomura, O. Ishida, and T. Ishii (1972): Studies on the seed production of the Japanese eel. III. Structure of the ovarian oocytes and ovulated eggs. Suisannzoshoku **19**, 201-209.

7) Yamamoto, K., M. Oomori, and K. Yamauchi (1974): Oogenesis of the Japanese eel. Nippon Suisan Gakkaishi, **40**, 9-15.

8) Kagawa, H., N. Iinuma, H. Tanaka, H. Ohta, and K. Okuzawa (1998): Effects of rearing period in seawater on induced maturation in female Japanese eel *Anguilla japonica*. Fisheries Science, **64**, 77-82.

9) Yamamoto, K., T. Morioka, O. Hiroi, and M. Oomori (1974): Artificial maturation of female Japanese eels by the injection of salmonid pituitary. Nippon Suisan Gakkaishi, **40**, 1-7.

10) Kagawa, H., H. Tanaka, H. Ohta, K. Okuzawa, and K. Hirose (1995): *In vitro* effects of 17**α**-hydroxyprogesterone and 17**α**,20**β**-dihydroxy-4-pregnen-3-one on final maturation of oocytes at various developmental stages in artificially matured Japanese eel *Anguilla japonica*. Fisheries Science, **61**, 1012-1015.

11) Ohta, H., H. Kagawa, H. Tanaka, K. Okuzawa, and K. Hirose (1996): Changes in fertilization and hatching rates with time after ovulation induced by 17,20**β**-dihydroxy-4-pregnen-3-one in the Japanese eel, *Anguilla japanica*. Aquaculture, **139**, 291-301.

12) Ohta, H., H. Kagawa, H. Tanaka, K. Okuzawa, N. Iinuma, and K. Hirose (1997): Artificial induction of maturation and fertilization in the Japanese eel, *Anguilla japonica*. Fish Physiology and Biochemistry, **17**, 163-169.

13) Kagawa, H., H. Tanaka, H. Ohta, K. Okuzawa, and N. Iinuma (1997): Induced ovulation of 17,20**β**-dihydroxy-4-pregnen-3-one in the artificially matured Japanese eel, with special reference to ovulation time. Fisheries Science, **63**, 356-367.

14) Ohta, H., H. Kagawa, H. Tanaka, K. Okuzawa, and K. Hirose (1996): Milt production in the Japanese eel *Anguilla japonica* induced by repeated injections of human chorionic gonadotropin. Fisheries Science, **62**, 44-49.

15) Ohta, H., K. Ikeda, H. Kagawa, H. Tanaka, and T. Unuma (2001): Acquisition and loss of potential for motility of spermatozoa of the Japanese eel Anguilla japonica. Proc. the 28th US-Japan Natural Resources Aquaculture Panel. (ed by C.C.-T.Tamaru, C.S. Tamaru, J.P. McVey, and K. Ikuta), **28**, pp. 77-81.

16) Ohta, H., K. Ikeda, and T. Izawa (1977): Increases of concentrations of potassium and bicarbonate ions promote acquisition of motility in vitro by Japanese eel spermatozoa. J. Exp. Zool., **277**, 71-80.

17) Tanaka, H., H. Kagawa, H. Ohta, K. Okuzawa, and K. Hirose (1995): The first report of eel larvae ingesting

rotifers. Fisheries Science, **61**, 171-172.
18）Kurokawa, H., H. Kagawa, H. Ohta, H. Tanaka, K. Okuzawa, and K. Hirose (1995): Development of digestive organs and feeding ability in larvae of Japanese eel (*Anguilla japonica*). Can. J. Fishe. Aquat. Sci., **52**, 1030-1036.
19）Kurokawa, H., H. Tanaka, H. Kagawa, and H. Ohta (1996): Absorption of protein molecules by the rectal cells in eel larvae *Anguilla japonica*. Fisheries Science, **62**, 832-833.
20）Tanaka, H., H. Kagawa, and H. Ohta (2001): Production of leptocephali of Japanese eel (*Anguilla japonica*) in captivity. Aquaculture, in press.

ウナギの種苗生産研究

香川浩彦・太田博巳・田中秀樹

ウナギは淡水養殖上，非常に重要な魚種で，日本の食文化に欠かせない食材である。しかし，ウナギ養殖の種苗となる天然シラスウナギ資源の減少や種苗価格の上昇から，人工種苗生産技術の開発が長年待ち望まれているにもかかわらず，未だに完成していない。ここでは我々の研究所で行っている雌雄のウナギの人為催熟技術や仔魚の飼育技術に関する研究について報告する。