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Citation	水産増殖, 58(2), 269-278
Issue Date	2010-06-20
Doc URL	<a href="https://hdl.handle.net/2115/47540">https://hdl.handle.net/2115/47540</a>
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Type	journal article
File Information	Suisanzoshoku58(2) pp. 269-278.pdf



## The Relationship between the Developmental Stage of Oocytes in Various Seasons and the Quality of the Egg Obtained by Artificial Maturation in the Feminized Japanese Eel *Anguilla japonica*

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**Abstract:** Feminized 2-year-old eels were reared in freshwater at 26°C until October, then the water temperature was gradually decreased to 16°C to December, and gradually increased to 26°C from February to April. They received weekly injections of salmon pituitary extract (body weight, 15 or 30 mg/kg) to induce sexual maturation in September, December, or April. The quality of the eggs obtained in each season was evaluated by the resulting fertilization, hatching, and 8-day survival rates. High quality eggs were obtained via high-dose injections in most experimental groups. In the high-dose injection groups, eels were induced to the final maturation phase in all seasons; however, egg quality obtained in April was extremely low in contrast to that obtained in September and December. Histological observation of oocytes just before the process of artificial maturation was commenced indicated that the developmental stages of oocytes varied by season: oil droplet in September, early vitellogenic in December, and regressive from vitellogenesis in April. The extremely low egg quality in April is probably due to the fact that the oocytes had already entered the regressive stage. These results indicated that better quality of eel eggs can be obtained by starting the process of artificial maturation between September and December.

**Key words:** Japanese eel; Artificial maturation; Egg quality; Oocyte development

The Japanese eel *Anguilla japonica* is one of the most important species of the aquaculture industry of East Asia. A supply of Japanese eel elvers for an aquaculture farm is completely dependent on a wild catch of glass eels. However, natural sources of glass eels have declined rapidly in recent years (Tatsukawa 2003). Therefore, it is necessary to establish a method for seed production of eel elvers that is not dependent on the wild catch. However, artificial production of elvers is very difficult in eels (Yamamoto et al. 1972, 1974). Yamamoto and Yamauchi (1974) first successfully induced sexual maturation of female eels and obtained fertilizable eggs (Yamamoto and Yamauchi 1974). Those eggs were used for artificial fertilization and the resulting larvae survived for

2 weeks (Yamauchi et al. 1976). After a long interval, Tanaka et al finally managed to produce glass eels in captivity (Tanaka et al. 2003), however, it is still extremely difficult to produce eel elvers under artificial conditions. One of the major problems of seed production is stable production of high quality eggs. Egg quality, in terms of resulting fertilization, hatching, and survival rates, is highly variable between eggs of different individuals. In most cases, larval survival rates are extremely low primarily due to poor egg quality. Thus, it is necessary to improve techniques to produce high quality eggs in order to make mass production of eel elvers possible.

Sexual maturation in eels was induced by weekly injections with salmon pituitary extract (SPE) using feminized, cultivated eels according

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Received February 23, 2010; Accepted April 19, 2010.

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to our regular method (Ohta et al. 1997). When oocytes reached the migratory nucleus stage (MNS) after approximately 8 to 15 SPE injections, final oocyte maturation (FOM, re-initiation of meiosis) and ovulation were induced by injection of  $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one (DHP). In this regular method, our experiences that eggs obtained by artificial maturation tend to be high-quality in winter but to be low-quality in spring and summer suggest the existence of an obvious annual rhythm in oocyte development under captive conditions. A relationship among ovarian developmental stage before starting artificial maturation, SPE injection dosage, and the success of eel maturation was reported in our previous study. (Ijiri et al. 1998). However, the previous study evaluated only the maturation success rate and not the resulting egg quality, in terms of fertilization, hatching, and survival rates. Further, the relationship between the annual changes in oocyte development and the resulting egg quality produced by artificial maturation and appropriate dosage of SPE injections has not yet to be clarified. This study was designed to investigate why eggs produced by artificial maturation in winter tended to be of higher quality and why artificial maturation in spring tended to fail. We also aimed to investigate the relationship between the developmental stage of oocytes just before starting artificial maturation and appropriate SPE injection dosages.

## Materials and Methods

### Experimental fish

In spring 2005, a group of glass eels purchased from commercial eel supplier was feminized

by feeding diets containing estradiol- $17\beta$  (10 mg/kg) for 5 months (Tachiki and Nakagawa 1993; Tachiki et al. 1997) at  $28^\circ\text{C}$ , and the eels were then cultured in a freshwater tank with a commercial diet for an additional year until the start of artificial maturation. Eels were reared under a natural photoperiod with controlled water temperature at about  $26^\circ\text{C}$ . These eels (body weight (BW),  $319 \pm 9.4$  g,  $n = 21$ ) received weekly injections of SPE from September 2006. Glass eels purchased in spring 2006 also received the same feminizing treatment and were reared under the same conditions. The water temperature was kept at about  $26^\circ\text{C}$  in summer, gradually decreased to about  $16^\circ\text{C}$  from October to December, and gradually increased to  $26^\circ\text{C}$  from February to April (Fig. 1). These eels were divided into 3 groups and they received SPE injections in September 2007 (BW,  $318 \pm 11.5$  g,  $n = 29$ ), December 2007 (BW,  $303 \pm 12.2$  g,  $n = 24$ ), and April 2008 (BW,  $422 \pm 16.0$  g,  $n = 21$ ), respectively.

### Ovarian developmental stage before SPE injections

One week before the SPE injections were administered, ovarian fragments of all the eels were collected by biopsy to assess their developmental stage. Collected ovarian fragments from each fish were immediately immersed in ice-cold eel Ringer (NaCl 151 mM, KCl 3.3 mM,  $\text{CaCl}_2$  4.9 mM,  $\text{MgCl}_2$  3.5 mM, HEPES 10 mM; pH 7.4) and were divided into 2 pieces. Ovarian follicles were isolated from an ovarian fragment with fine forceps, and the diameters of 100 randomly selected oocytes were measured. The diameter of the largest group of oocytes, which was considered the oocyte diameter of the experimental

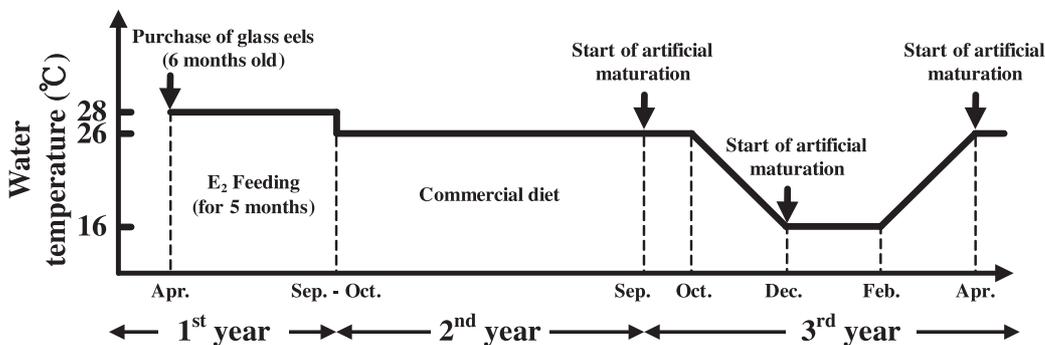


Fig. 1. Schematic representation of the rearing conditions.

eel, was calculated by averaging the 20 largest oocyte diameters. At the same time, the other piece of ovarian fragment was fixed with Bouin's fixative, embedded in paraffin and cut at a  $5\ \mu\text{m}$  thickness. The sections were stained with hematoxylin and eosin or alternatively immunostained with the anti-serum against eel vitellogenin (eel ant-VTG) (Okumura et al. 2001).

#### *Artificial maturation*

After the biopsy was conducted, the eels were gradually acclimated to the seawater over the course of 1 week. Eels were maintained without feeding in dark tanks at a constant water temperature of  $20^\circ\text{C}$ . In each experiment, eels were randomly divided into 2 sub-groups and respectively received weekly intraperitoneal SPE injections of 15 (low-dose) or 30 (high-dose) mg of acetone-dried pituitary powder/kg BW. The number of eels in each experimental group is shown in Table 1. After approximately 7 to 17 injections of SPE, oocytes completed vitellogenesis, and migration of each nucleus was spontaneously initiated. When the BW of eels increased by 110% compared to the initial BW, a biopsy of the ovarian fragments was conducted and oocyte diameters were measured. If the oocyte diameter was more than  $800\ \mu\text{m}$ , an additional SPE injection was administered as a "priming injection" at the same dose as the weekly injections to enhance maturational competency, which promotes FOM in response to DHP injections. At 24 h after the priming injection, DHP (Steroids Inc., Newport, RI, USA) was injected at 2 mg/kg BW to induce FOM and ovulation. At the same schedule, spermatogenesis was induced in male eels via weekly injections of human chorionic gonadotropin (hCG; Sankyo Yell Yakuhin CO., Ltd., Tokyo, Japan) at a dose of 1 IU/g BW for over 6 weeks, then spermiation was induced via an injection of DHP (1 mg/kg BW) (Ohta et al. 1996).

#### *Artificial fertilization of eggs and rearing of larvae*

In case of ovulation at approximately 15 h after the DHP injection, eggs were immediately collected by pressing the abdomen of the eel. Then, 2 g of eggs were inseminated with 1 ml of

diluted milt, which was diluted (50 x) by artificial eel seminal fluid (NaCl 114.5 mM, KCl 30 mM,  $\text{CaCl}_2$  1.3 mM,  $\text{MgCl}_2$  1.6 mM,  $\text{NaHCO}_3$  20 mM; pH 8.2), and then transferred to 100 ml of filtered natural seawater. A 1-ml suspension containing about 100 to 200 eggs was transferred into a glass dish and only buoyant eggs were transferred to two 48-well culture plates (96 buoyant eggs); one egg was placed in one well. Each well was filled with 1 ml of filtered natural seawater (containing 35 mg/l penicillin and 50 mg/l streptomycin). The eggs were then incubated at  $23^\circ\text{C}$ . Fertilization, hatching, and survival rates of the larvae 8 days post hatching (DPH) were examined by use of an individual rearing method detailed by Unuma et al. (Unuma et al. 2004). Fertilization, hatching, and survival rates were calculated by the following formula:

Fertilization (hatching or survival) rate (%) =  $100 \times (\text{buoyancy rate} \times \text{fertilization (hatching or survival) rate})$

#### *Statistical analyses*

Data are presented as means  $\pm$  standard error of the mean (SEM). Data for oocyte diameter, fertilization rate, hatching rate, and survival rate were analyzed by a one-way ANOVA followed by Duncan's test (software SPSS 13.0). Statistical significance was accepted at  $P < 0.05$ .

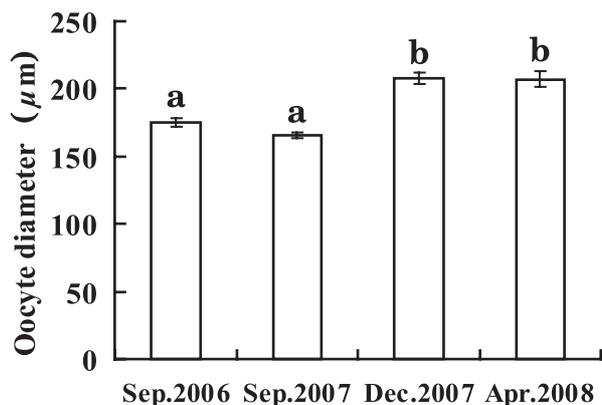
## Results

#### *Oocyte diameters before starting SPE injections*

Oocyte diameters before the SPE injections were administered were investigated in this study. The oocyte diameters were  $175 \pm 2.9\ \mu\text{m}$  in September 2006,  $166 \pm 2.2\ \mu\text{m}$  in September 2007,  $208 \pm 4.1\ \mu\text{m}$  in December 2007, and  $207 \pm 6.0\ \mu\text{m}$  in April 2008 (Fig. 2). The oocyte diameters measured in September 2006 and September 2007 were significantly smaller than those measured in December 2007 and April 2008 ( $P < 0.05$ ).

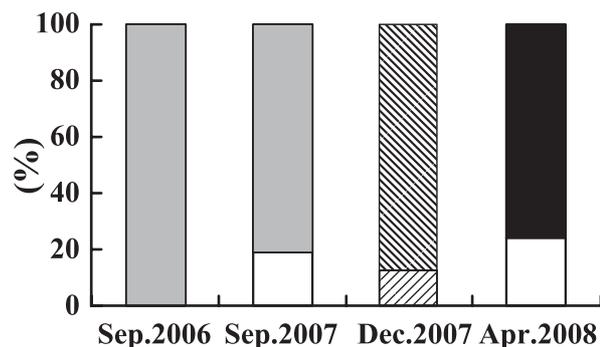
#### *Ovarian developmental stage before starting SPE injections*

Ovarian developmental stages before SPE injection were analyzed by histological observation.



**Fig. 2.** Oocyte diameters of eels before starting salmon pituitary extract (SPE) injections in September 2006 ( $n = 22$ ), September 2007 ( $n = 29$ ), December 2007 ( $n = 24$ ) and April 2008 ( $n = 21$ ). Each value represents the mean  $\pm$  SEM ( $P < 0.05$ ).

All 22 eels in September 2006 contained oocytes at the late oil droplet stage (Fig. 3). Many oil droplets were observed in oocyte cytoplasm but yolk globules were not detected by immunohistochemical observation against eel vitellogenin (Figs. 4B, 4b). Among 29 eels that were induced sexual maturation in September 2007, 6 fish (20.7%) contained relatively small oocytes at the early oil droplet stage (Fig. 3). Relatively fewer oil droplets were observed in the cytoplasm of these oocytes and no yolk globules were detected (Figs. 4A, 4a). The other 23 fish (79.3%) contained oocytes at the late oil droplet stage as seen in September 2006 (Figs. 3, 4B, 4b). All 24 eels in December 2007 contained oocytes at the early vitellogenic stage (Fig. 3). Oil droplets were filled in the oocyte cytoplasm and yolk globules appeared at the oocyte periphery, meanwhile, a few regressive oocytes were observed in several eels (Figs. 4C, 4D, 4c, 4d). Although the ovaries of all the 24 eels were at the early vitellogenic stage, 3 fish (12.5%) contained oocytes at the beginning stage of yolk globule accumulation. The number of yolk globules in these 3 fish was obviously less than the other 21 fish (87.5%). Among the 21 female fish in April 2008, 16 fish (76.2%) contained ovaries at the regressive stage (Fig. 3). The regressive oocytes (the largest group) contained yolk globules at their periphery while the residual small oocytes (the second-largest group) were still at the early oil droplet stage (Figs. 4E, 4e). The



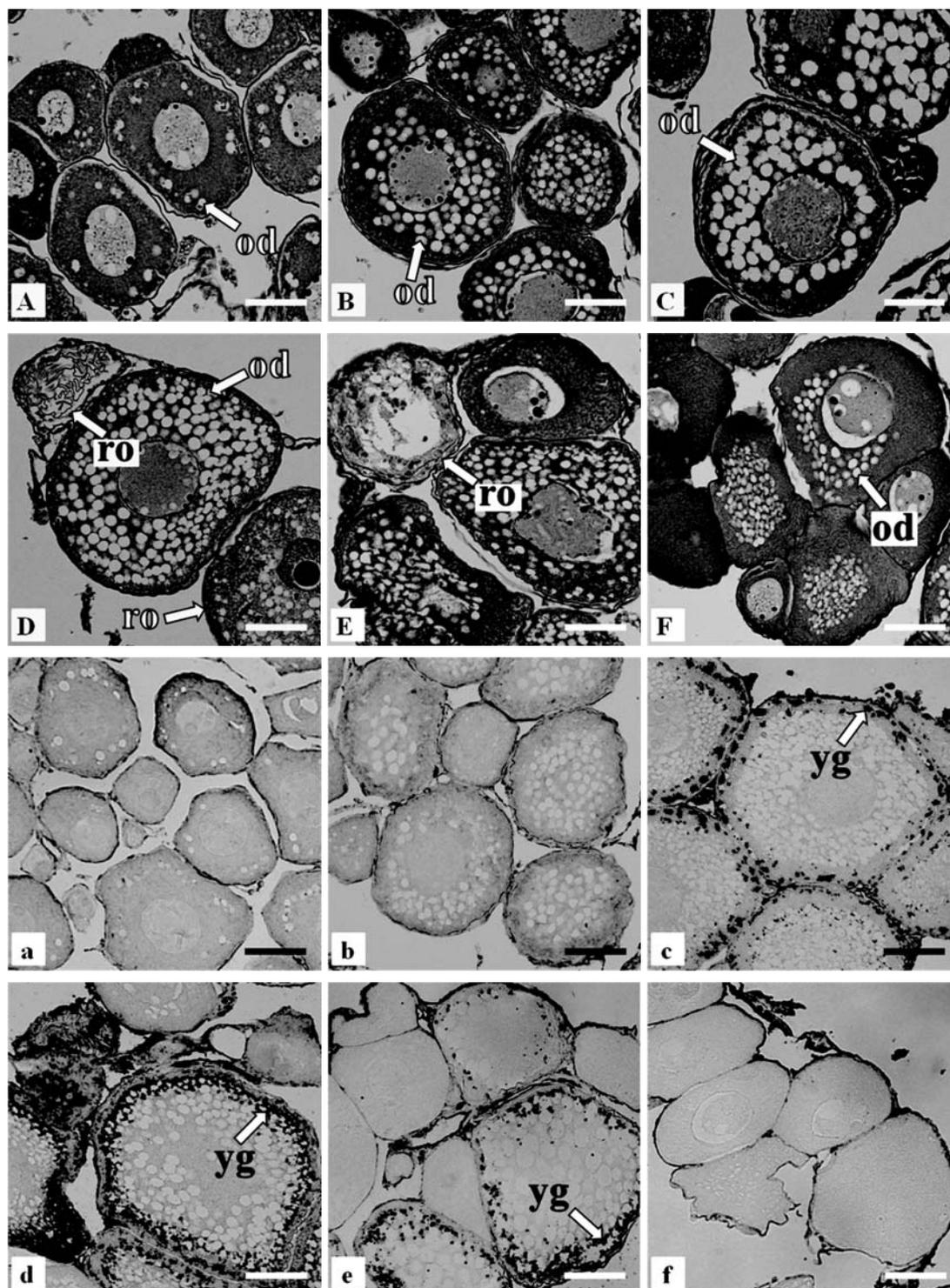
**Fig. 3.** Oocyte developmental stages of eels in September 2006 ( $n = 22$ ), September 2007 ( $n = 29$ ), December 2007 ( $n = 24$ ) and April 2008 ( $n = 21$ ) before starting salmon pituitary extract (SPE) injections. □, Early oil droplet stage; ▒, Late oil droplet stage; ▨, Beginning stage of yolk globule accumulation; ▩, Early vitellogenic stage; ■, Regressive stage.

other 5 fish (23.8%) contained oocytes at the oil droplet stage in which oocytes were as small as those seen in September and no regressive oocytes were observed (Figs. 3, 4F, 4f).

#### *Induction of maturation and the number of SPE injections required to reach the migratory nucleus stage*

The number of eels whose oocytes reached the migratory nucleus stage (MNS) by artificial maturation was less in the low-dose group of SPE injection group with the exception of that in September 2006 (Table 1). The numbers of mature fish in low-dose treatment group and reached MNS were 11 (73.3%) of the 15 eels in September 2007, 8 (66.7%) of the 12 eels in December 2007, and 5 (45.5%) of the 11 eels in April 2008, respectively (Table 1). At the same time, all eels treated with high-dose SPE injections reached MNS in both September 2006 and December 2007 (Table 1). Of the 14 eels treated in September 2007, 12 fish (85.7%) reached MNS. Of 10 eels in April 2008, the numbers of mature fish was 7 (70.0%) (Table 1).

Eels reached MNS in the shortest period of time in the experiment started in September 2006. Eels treated with low-dose SPE required 8–11 injections, while eels in the high-dose group required 7–11 injections to reach MNS (Figs. 5A, 5B). The relationship between the initial oocyte diameter and the number of SPE injections required to reach MNS did not



**Fig. 4.** Photomicrographs of Japanese eel ovaries before starting salmon pituitary extract (SPE) injections. Ovarian sections stained with hematoxylin and eosin (A–F), or immunostained with eel anti-vitellogenin (a–f). (A, a), early oil droplet stage (September); (B, b), late oil droplet stage (September); (C, c), beginning stage of yolk globule accumulation (December); (D, d), early vitellogenic stage (December); (E, e), regressive stage (April); (F, f), early oil droplet stage (April). od, oil droplet; yg, yolk globule; ro, regressive oocyte. Bars = 50  $\mu$ m.

show a significant positive correlation. In the experiment started in September 2007, eels required more injections (11–16 low-dose injections and 8–17 high-dose injections) than that in September 2006, and eels with small initial

oocyte diameters tended to require more injections to reach MNS in both the low- and high-dose groups (Figs. 5C, 5D). In the experiments started in December 2007 and April 2008, the relationship between initial oocyte diameter and

**Table 1.** The results of artificial maturation induced by low-dose or high-dose SPE injections

Start of SPE injections	SPE injection dose	Number of fish <sup>1</sup>	Maturation rate (%) <sup>2</sup>	Number of injections <sup>3</sup>	Oocyte diameter before SPE injections ( $\mu\text{m}$ ) <sup>4</sup>	
					Mature fish	Immature fish
Sep. 2006	Low-dose	11 (11/0)	100	9.6 $\pm$ 0.4	175 $\pm$ 4.4 <i>max</i> = 203, <i>min</i> = 156	—
Sep. 2007		15 (11/4)	73.3	14.5 $\pm$ 0.5	166 $\pm$ 3.7 <i>max</i> = 149, <i>min</i> = 183	164 $\pm$ 7.6 <i>max</i> = 177, <i>min</i> = 142
Dec. 2007		12 (8/4)	66.7	12.0 $\pm$ 0.7	213 $\pm$ 7.9 <i>max</i> = 179, <i>min</i> = 242	19.5 $\pm$ 8.0 <i>max</i> = 214, <i>min</i> = 176
Apr. 2008		11 (5/6)	45.5	12.8 $\pm$ 0.8	205 $\pm$ 9.6 <i>max</i> = 182, <i>min</i> = 233	207 $\pm$ 12.4 <i>max</i> = 259, <i>min</i> = 175
Sep. 2006	High-dose	11 (11/0)	100	8.7 $\pm$ 0.5	176 $\pm$ 4.1 <i>max</i> = 159, <i>min</i> = 201	—
Sep. 2007		14 (12/2)	85.7	10.7 $\pm$ 0.8	169 $\pm$ 3.1 <i>max</i> = 151, <i>min</i> = 186	151, 153
Dec. 2007		12 (12/0)	100	8.8 $\pm$ 0.5	210 $\pm$ 5.5 <i>max</i> = 182, <i>min</i> = 245	—
Apr. 2008		10 (7/3)	70.0	11.0 $\pm$ 0.6	199 $\pm$ 11.4 <i>max</i> = 172, <i>min</i> = 252	233 $\pm$ 8.8 <i>max</i> = 251, <i>min</i> = 224

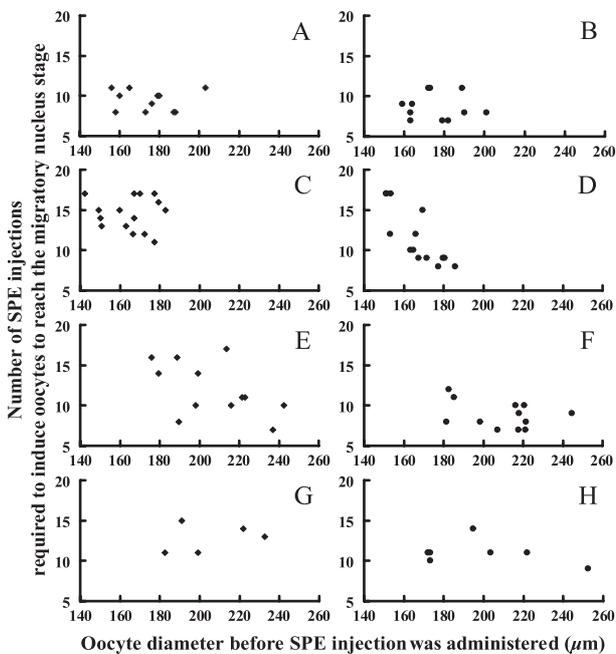
<sup>1</sup>The number of total treated fish (mature fish which reached MNS / immature fish).

<sup>2</sup>Maturation rate (100 %) = number of matured fish  $\div$  number of total treated fish  $\times$  100.

<sup>3</sup>Number of injections required to induce female fish to attain final oocyte maturation phase (each value represents the mean  $\pm$  SEM).

<sup>4</sup>Oocyte diameter of mature fish and immature fish before SPE injections were administered ( $\mu\text{m}$ ).

—, No fish failed to induce to maturation phase.



**Fig. 5.** The relationship between oocyte diameter before starting hormonal induction and the number of SPE injections required to induce oocytes to reach migratory nucleus stage (MNS). Low-dose SPE treated eels in September 2006 (A), September 2007 (C), December 2007 (E), April 2008 (G), and high-dose SPE treated eels in September 2006 (B), September 2007 (D), December 2007 (F), April 2008 (H).

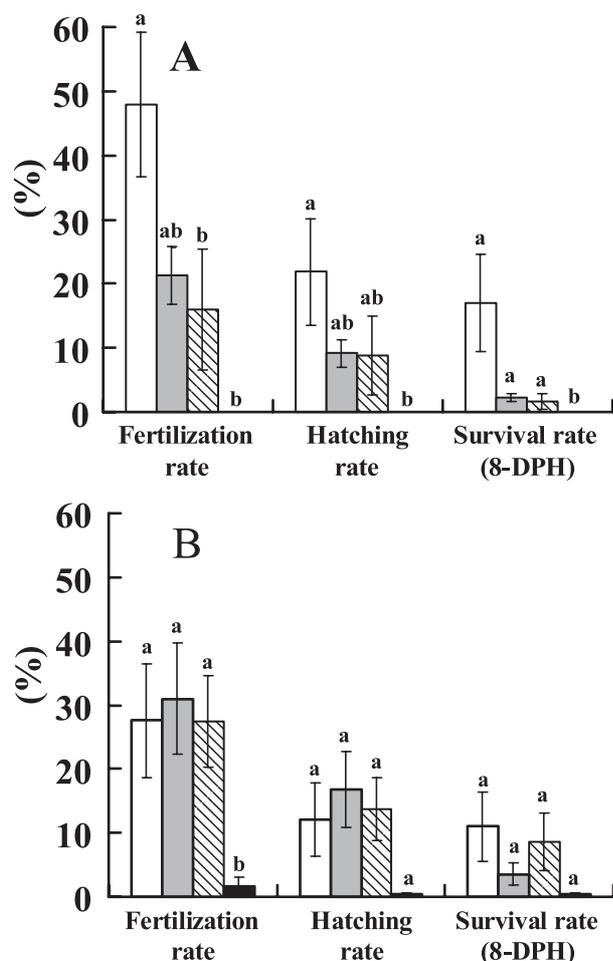
the number of SPE injections needed to reach MNS did not show a positive correlation (Figs.

5E, 5F, 5G, 5H). In the experiment started in December 2007, eels required 7–14 low-dose SPE injections or 7–12 high-dose SPE injections to reach MNS (Figs. 5E, 5F). In the experiment started in April 2008, eels in the low-dose group required 11–15 injections and eels in the high-dose group required 9–11 injections (Figs. 5G, 5H).

#### *Egg qualities evaluated by fertilization, hatching and survival rates*

In the low-dose SPE injection groups, the highest egg quality was obtained in September 2006. The fertilization, hatching and survival rates were 47.9%, 21.9% and 17.0%, respectively. In September 2007, those rates were 21.3%, 9.1% and 2.2%, respectively. Those rates in December 2007 were 16.0%, 8.8% and 1.5%, respectively. On the other hand, no eels were induced to final maturation in April 2008 (Fig. 6A).

In the high-dose injection groups, in the experiment started in September 2006, fertilization, hatching and survival rates were 27.6%, 12.1% and 11.0%, respectively. In the experiment started in September 2007, these ratios were 33.8%, 16.8%, and 3.5%, respectively. Relatively



**Fig. 6.** The fertilization, hatching, and survival rates 8 days post hatching (8-DPH) in September 2006 (□), September 2007 (■), December 2007 (▨) and April 2008 (■). Low-dose induction (A), high-dose induction (B). Each value represents the mean  $\pm$  SEM ( $P < 0.05$ ).

lower results were obtained in the experiment in December 2007, yielding a fertilization rate of 27.4%, hatching rate of 13.7%, and survival rate of 8.6%. The worst result was found in the experiment started in April 2008, in which the fertilization rate was 4.9% and the hatching and survival rates were 0.9% each (Fig. 6B).

## Discussion

Our previous report indicated that eels whose oocyte follicle diameter was greater than  $180 \mu\text{m}$  could be induced to the final maturational phase and most feminized eels used in the report fell within that range (Ijiri et al. 1998). In the present study, we determined the best period in the year for starting artificial maturation in order to obtain high-quality eggs and demonstrated

a physiological background which determined success ratio of artificial maturation.

In our usual rearing conditions, glass eels purchased in late winter to early spring were reared in fresh water at  $28^\circ\text{C}$  and were induced to female differentiation by estrogen feeding for 5 months. In the next year, oocytes of those eels had progressed to the oil droplet stage and those follicular diameters reached 150 to  $200 \mu\text{m}$  (average  $175 \mu\text{m}$ ) in September. At this oocyte developmental stage, most of the eels were induced by SPE injections to the final maturational phase (oocyte in the migratory nucleus stage) and re-initiation of meiosis followed ovulation by DHP injection. In the wild, Japanese eels are thought to spawn from April to November with a peak in July around the West Mariana Ridge (Tsukamoto et al. 2003). Eel larvae can drift for over 6 months after birth to the Japan sea shore, after which time they up-migrate to the river as glass eels (Kimura 2003). At this stage, glass eels are around 6 months old (Tzeng 1985; Tsukamoto 1990); hence, the eels in September of the second year after capture were thought to be around 2 years old. Under rearing conditions employed in this study, eels that are about 2 years old can be used as a parent fish for artificial maturation and relatively high-quality eggs are obtained by SPE injections, which are started as early as September.

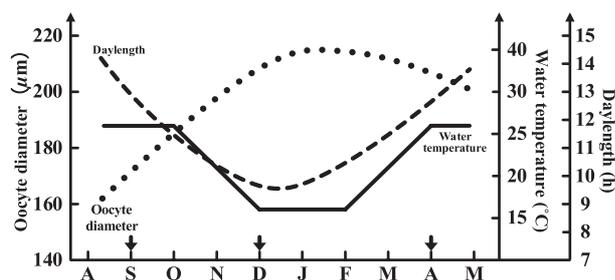
This study examined the effects of high-dose ( $30 \text{ mg/kg-BW}$ ) or low-dose ( $15 \text{ mg/kg-BW}$ ) SPE injections on artificial maturation. Overall, the ratio of eels that entered the final maturational phase was higher in the high-dose groups. In either group, most eels could be induced to the final maturational phase in September (the follicular diameter of the largest group being as small as  $150 \mu\text{m}$ ) or December (the average diameter being at least  $180 \mu\text{m}$ ). The ratios of eels reaching the final maturational phase were very high in both groups. These observations indicate that 2-year-old feminized eels with follicular diameters as small as  $150 \mu\text{m}$  could be used for artificial maturation purposes from September to December. In contrast, the two eels that were not induced to the final maturational phase by high-dose injections

in September 2007 had follicle diameters of  $150\ \mu\text{m}$  before treatment. This diameter is not small when compared with diameter in other eels that were successfully induced to the final maturational phase. This suggested that some eels were not induced to the final maturational phase due to not only developmental stage of the oocytes before treatment but also other factors, for example, immunoreactions to SPE, reduction of GTH receptors in the follicle and/or injection stress. This problem was also discussed in our previous report (Ijiri et al. 1998). Unlike the conclusion of this study, our previous study suggested that  $180\ \mu\text{m}$  was the lower limit of follicle diameter before treatment for successful induction by artificial maturation. However, the estimated mean in the study had been derived from experiments in comparison of feminized cultivated eels and normal cultivated eels that were not artificially feminized (not treated with estradiol- $17\beta$  during glass eel stage). In fact, normally cultivated eels that naturally differentiated to females required follicular diameters of greater than  $180\ \mu\text{m}$  for successful artificial maturation; however, feminized eels in the present study had follicular diameters as small as  $150\ \mu\text{m}$  and were still induced to the final maturational phase. This fact may indicate that estrogen treatment of glass eels influences not only eel feminization but also subsequent ovarian development.

Although the ratio of individuals reaching the final maturational phase by SPE treatment started in April was low in comparison to the experimental groups in September and December, the ratio of the eels that were not induced to the maturational phase was not high in the high-dose treatment group. Indeed, about 70% of eels were induced to the final maturational phase in that group in April. However, egg quality regarding fertilization, hatching was fatal in the experimental group in April. High quality eggs were not obtained on April at all but were obtained in September and December. No significant differences in maturation, fertilization, hatching rates were found between September and December. In addition, the ratios of larval hatching and

survival were higher in the high-dose treatment groups except for the experiment in September 2006, in which higher-quality eggs were obtained via low-dose treatment. This case was the only example in which low-dose treatment was superior to high-dose treatment. This may have been caused by intra-follicular overripe of oocytes without undergoing germinal vesicle breakdown. In these eels, oocyte development may progress relatively quickly in the migratory nucleus stage after the priming injection (the additional injection of SPE following regular weekly injections), and a regular schedule of DHP injection might miss the appropriate timing for triggering the final oocyte maturation. These oocytes might reach pre-maturation (intra-follicular overripe) prior to DHP injection. This is no more than speculation; however, this case may suggest that low-dose treatment may enable prevention of intra-follicular pre-maturation before DHP injection in case of using eels whose responses to SPE injection are relatively sensitive. This may be a future issue to examine for improving artificial maturation techniques. Taking together, considering the ratio of individuals reaching the final maturational phase and the resultant egg quality, the period from September to December is the best time to start artificial maturation. Beyond December, oocytes on December tend to show the beginning of regression.

The average diameter of the most advanced group of ovarian follicles had advanced between September and December. Oocytes in the oil droplet stage in September had started vitellogenic growth by December. It is suggested that dropping temperatures between October and December may induce natural vitellogenic growth in this study (Fig. 7). In the case of eels that were reared at a constant temperature at  $26^\circ\text{C}$  even after October, oocytes that were progressed to the vitellogenic growth spontaneously were not observed (manuscript in preparation). Although a small accumulation of yolk globules was observed (initial phase of vitellogenic growth) in some eels in the constant condition at  $26^\circ\text{C}$ , the present study was the first case in which oocytes that had advanced



**Fig. 7.** Schematic representation of oocyte diameters in eels and the environmental conditions. Changes in oocyte diameter, water temperature, and day length based on the information released by the National Astronomical Observatory of Japan. Arrows indicate the time when salmon pituitary extract (SPE) injections were started.

to the early vitellogenic stage were observed in all eels under captive conditions. On the other hand, the averaged follicle diameter in April was equal to that in December. However, histological features in April were quite different from those in December. In contrast to histological observation in December of the early vitellogenic oocytes, those oocytes had advanced to the regressive stage in April. This regression in vitellogenic oocytes may be triggered by either elevating temperature after February or lengthened daylight after the end of December. Most obviously, the reason why high-quality eggs were not obtained by artificial maturation after spring is probably due to the advancing regressive process of vitellogenic oocytes after December. Notably, if vitellogenic oocytes in December may be protected from the regressive process by environmental manipulation such as temperature or lighting conditions, it would be a very useful technique for providing parent eels for artificial maturation throughout the year. This encouraging possibility should be examined in future studies.

In summary, we conclude that the appropriate time period to start artificial maturation in 2-year-old feminized eels is September to December. The reason that high-quality eggs are hardly obtained after spring is the advancement of the regressive process of vitellogenic oocytes in this period. Further study is necessary to establish a technique that enables maintenance of vitellogenic oocytes without advancing regression by environmental manipulation in order

to help parent eels produce high-quality eggs throughout the year.

## Acknowledgments

We thank the colleagues of the Japanese eel research team of our laboratory for their encouragements and helps. This study was supported in part by Grant-in-Aid for 21st century COE Program (to S.A. and K.Y.), Grant-in-Aid for Scientific Research from the Ministry of Education, and Grant-in-Aid from the Ministry of Agriculture, Forestry and Fisheries, Japan.

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## ニホンウナギの人為催熟における卵質の季節変化

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雌化ウナギ2歳魚を9月、12月または4月にサケ脳下垂体注射によって人為催熟し、得られる卵質の差を受精率、孵化率および8日後生存率で評価した。9月、12月は比較的良質な卵が得られる傾向が認められたが、4月では卵は得られるものの、その卵質は極めて悪かった。催熟前の卵母細胞を組織学的に観察すると、12月に卵黄形成開始が観察されたが、4月には退行している様子が観察された。4月に催熟を開始しても良質な卵が得られない原因として、催熟前に既に卵母細胞が退行しつつあることが考えられた。以上の結果から、雌化ウナギ2歳魚の人為催熟は9月から12月の間にかけて開始することが良質な卵を得るために適当であると結論された。