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REVIEW ARTICLE

Progression in artificial seedling production of Japanese eel *Anguilla japonica*

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Abstract Eel aquaculture, though thriving nowadays, is totally dependent on the successful capture of wild eel fry and glass eels for its seedlings. The declination of eel resources in recent years has resulted in an urgent need for technology development in artificial seedlings production on an aquaculture basis, in order to protect natural resources and to stabilize the eel supply in the farming industry. Since the life history of the eel holds many mysteries, artificial hatching and rearing of larvae has long been regarded as an extremely difficult task. However, in recent years, the spawning ground of the Japanese eel has finally been located after continuous effort with intensive marine surveys, in which wild parental eels were captured, followed by the collection of fertilized eggs and the harvest of newly hatched preleptocephali. Meanwhile, through the collaborative efforts of many researchers, progress has also been made in improving technologies for artificial maturation of parental eels, which do not mature naturally in captivity, as well as in the technology for artificial hatching. Moreover, a technology for producing feed-rearing eel hatchlings, the most challenging process of all, has advanced rapidly after suitable feed was developed in the 1990s. Then, in 2002, for the first time in the world, larvae were successfully reared up to the glass eel stage, and second generation artificial hatchlings were born in 2010. In this way, eel farming technology that is not reliant on natural resources has been developed. There are strong hopes now for a technology for stable mass production of glass eels to be developed in the near future.

Keywords *Anguilla japonica* · Fry production · Glass eel · Japanese eel · Larval rearing · Leptocephalus · Metamorphosis

Introduction

It is said that the Japanese consume around 40,000 tons of eel every year. More than 99 % are farmed eels reared from fry known as glass eels, which are caught in the wild. Although full-life cycle culture from egg to adult is now possible in many fish species, it has never been successfully accomplished in eels, despite arduous efforts for many years. Especially because it is still impossible to rear eels from egg to fry on a practical basis, collecting wild fry remains indispensable in eel farming.

The problem with this, however, is that catches of glass eels fluctuate wildly from year to year, and resources are in a long-term declining trend. In fact, catches have been extremely poor since 2010 [Fisheries Agency website, http://www.jfa.maff.go.jp/j/study/saibai/pdf/130826siryou_1.pdf (in Japanese) accessed 14 Apr 2014]. The decline of eel resources has become a serious problem, not only in Japanese eel, which are the most intensively cultured and developed in the eel-farming industry [1], but also in European and American eels [2, 3]. This has been attributed to a number of causes, including changes in the oceanic environment due to global warming, the loss of freshwater habitats, obstructions such as weirs and dams, and pollution in estuaries and coastal areas. Besides these, the excessive capture of glass eels for aquaculture is feared to have a major impact on resources [1, 4]. In light of these

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situations, there are now strong calls for the development of an artificial seedling production technology and the attainment of full-life cycle culture (whereby the life cycle from egg to parent can be completed in captivity), to protect eel resources and stabilize the eel farming industry.

Since the end of the 1980s, the author and others at the National Research Institute of Aquaculture (NRIA) of the Fisheries Research Agency (FRA) have been attempting to improve technologies for maturity inducement in parental eels and artificial insemination, based on many achievements by previous researchers [5–7]. This has realized the production of hatched larvae of rather good quality, in fairly large volume and on a systematic basis [8, 9]. Meanwhile, with the development of feed that is well eaten by artificial hatchling, feed-based rearing has become possible [9, 10], and in 2002, for the first time in the world, we succeeded in growing artificially hatched larvae to the glass eel stage [11, 12]. Since then, nearly all researchers connected with eel research in Japan, including those at the FRA and prefectural fisheries research institutes, universities, etc., have joined forces in tackling challenges in the technological development of cultivation and maturation of parental eels, feed for larvae, and optimization of the rearing environment. All these efforts culminated in successful rearing of artificially hatched eels, linked to the production of the next generation, whereupon "full-life cycle culture" was achieved in the spring of 2010.

In this paper, the author introduces the research progression of artificial seedling production of the Japanese eel, with particular emphasis on a key challenge: the development of larval rearing technology.

Information gleaned from research on life history

No report has been found on the eel's maturing and spawning ecology in nature, whatever their age or size, whether in cultivation containers or in rivers, lakes, coastal waters or other places close to human presence. Male farmed eels are thought to maintain an extremely low gonadosomatic index [GSI = (gonad weight)/(body weight) × 100, a measure of maturity] of less than around 0.2 throughout the year, with no marked seasonal variation [13]. Conversely, female farmed eels undergo slight gonad maturation from autumn to winter, with a GSI of around 2 in notable cases, and the ovarian eggs develop from the oil droplet stage to the primary yolk stage. From spring onwards, however, eggs in the yolk stage regress and the GSI also returns to a lower level [13].

By contrast, some wild downstream-migrating female eels with more advanced maturity are known to appear in lower reaches of large rivers, brackish lakes, coastal waters and elsewhere from autumn to early winter. In Mikawa Bay and near Kamishima Island at the mouth of Ise Bay, catches of downstream-migrating eels with a GSI of up to 3–4 in winter and with eggs in the secondary yolk stage have been reported. But the individuals at this maturing stage disappear by February or March, and only immature individuals are caught between April and August [14]. After starting to mature in winter, downstream-migrating eels are thought to mature progressively during the migration period until they arrive at the spawning ground. Until just recently, however, there had been no records of eels caught during spawning migration in open seas south of the Kuroshio current, and thus no detailed information had been obtained on the progression of maturity in wild eels.

The mystery of where eels are born and how they move to our coasts has occupied the minds of scholars through generations. In the case of European eels, at the end of the 19th century, Italians Grassi and Calandruccio [15] confirmed that the strange transparent fish shaped like willow leaves caught in the Mediterranean, called "leptocephali", were in fact eel larvae. In the 20th century, research by Schmidt [16] proved that the spawning ground of Atlantic eels was in the Sargasso Sea. Research on the marine life phase of the Japanese eel distributed in East Asia began in earnest in the 1970s, and in 1991 Katsumi Tsukamoto and other researchers of the University of Tokyo estimated that the spawning ground of Japanese eel was located near 15°N, 141–143°E, to the west of Guam Island [17].

Later, researchers mainly from the University of Tokyo, and the FRA, as well as others, continued surveys in seas around the Marianas. All these efforts led to a succession of "world firsts" in recent years: 2–5-day-old pre-feeding larvae (preleptocephali) were collected in 2005 [18], post-spawning parental eels were captured in 2008 and 2009 [19], and in 2009, fertilized eggs were collected [20]. These surveys have provided information on the physical and biological environments of eel spawning grounds, as well as the nature of mature parental eels and fertilized eggs, the shape of preleptocephali, the gut content and growth process of wild leptocephali, etc. [17–20]. This information provides very important guidelines for researching seedling production, which until these recent developments had been pursued in an altogether hit-or-miss fashion.

History of research on artificial seedling production

In Japan, research on maturity inducement and artificial hatching of eels started in the early 1960s, and thus has a history of more than half a century. Since eels in captivity do not mature and spawn naturally, technology to artificially induce maturity had to first be developed. Maturity in fish, as in other vertebrates, is controlled by gonadotropic hormones secreted from the pituitary gland, and eels are

no exception to this. Normally, it would be ideal if the eels themselves could secrete gonadotropic hormones. However, the pituitary glands of immature eels do not secrete a sufficient quantity of gonadotropic hormones, and therefore artificial hormone treatment is essential for inducing maturity in eels.

For males, in 1960, Hibiya [21] of the University of Tokyo attempted to induce maturity by administering a hormone preparation mixed from mammalian pituitary and chorionic gonadotropin hormone, and in January of the following year, succeeded in collecting sperm. Maturity inducement technology in females took another ten or more years to be developed; it was not until the 1970s that Ishida and Ishii [22] of the Chiba Prefectural Fisheries Research Center, Fresh-Water Station, Yamamoto et al. [23] of Hokkaido University, and others successfully induced ovulation. Then, in 1973, Yamamoto and Yamauchi [5] of Hokkaido University succeeded in collecting matured eggs and sperm by injecting salmonid pituitary extract into downstream-migrating female eels and Synahorin (a hormone preparation mixed from pituitary gland and chorionic gonadotropin hormone) into downstream-migrating and farmed male eels. With this success, artificial hatching was achieved for the first time in the world, and the development of hatchlings was observed over 5 days after hatching. In 1976, Yamauchi et al. [6] of Hokkaido University reported on larvae development over the 14 days after hatching.

However, with the method of maturity inducement used at that time, eggs capable of being inseminated were rarely ovulated, and despite advanced maturity, they often ended in overripeness without being ovulated. A research by Yamauchi and Miura [24] in 1988 clarified that this was due to the fact that 17α,20β-dihydroxy-4-pregnen-3-one (DHP), a steroid hormone required for final oocyte maturation, was not secreted. As a result, successful ovulation induction became much surer than before by administering 1 μg/g body weight of DHP to female eels matured by repeated injections of salmon pituitary extract.

Meanwhile, in the research conducted on maturity inducement using downstream-migrating eels, it was very difficult not only to collect parental eels, which are obtainable in very limited seasons, but also to obtain those of good quality in large numbers. The idea of using farmed eels as parental eels was thus considered, but farmed eels are overwhelmingly male in number [25], and moreover, male and female eels are indistinguishable in appearance. Thus, securing female parental eels seemed to be a problem. To address this, from the second half of the 1980s, Tachiki et al. [26] of the Fresh-Water Branch of the Aichi Fisheries Research Institute attempted to develop a technology for rearing farmed parental female eels for spawning, and in 1991, developed a method of orally administering estrogen (estradiol-17β) to glass eels to induce feminization. Then the maturity of feminized females was induced after about 2 years and 6 months by administering hormones, followed by successfully obtaining hatched larvae [27].

With these technical innovations, opportunities to conduct a variety of experiments have increased dramatically in the research on artificial maturation and hatching of eels. Since the 1990s, these researches have led to the development of technology for obtaining good quality eggs and larvae on a stable basis, as well as larval rearing technology.

Development of technology for obtaining good-quality eggs on a stable basis

At NRIA, various technical conditions for inducing maturity in eels were studied. After the development of oocytes of feminized farmed eels that received repeated administrations of salmon pituitary extract, oocytes of different diameter groups ranging from 600 to 900 μm were cultured in vitro together with 1–100 ng/ml of DHP. The purpose of this was to clarify the most effective stage for administering DHP. As a result, it was revealed that final oocyte maturation and ovulation in females could be induced with high efficiency when using oocytes of diameters of >800 μm [28]. It was also proved that ovulation occurs at a fixed point in time after the administration of DHP (around 18 h), and that the timing of ovulation can be controlled by changing the timing of administration [29]. Until then, DHP had normally been administered in the morning to induce final oocyte maturation, and so ovulation had occurred between the middle of the night and early the next morning. But now, applying the above facts, eggs can be collected at around noon the day after an evening administration of DHP.

For males, administering human chorionic gonadotropin (hCG) had been shown to promote spermiation, but no details were known (such as the amount of milt obtainable or the motility of the sperm). Research at NRIA revealed that about 1 g of milt could be collected by injecting completely immature farmed eels (body weight about 250 g) with 1 IU of hCG per 1 g of body weight at least ten times at 7-day intervals [30]. However, there were some problems in this protocol. It is occasionally observed that the volume of milt is small in comparison to the size of testes, and the sperm have low motility in the males matured by this protocol. Since the pH of the fluid (seminal plasma) surrounding the sperm and various ion concentrations were thought to be important in obtaining the motility of fish sperm, a detailed study of these made it clear that potassium and bicarbonate ions are important in obtaining and maintaining the motility of eel sperm [31]. Thus, artificial seminal plasma containing a suitable concentration of these

ions was prepared, and it was proved that when sperm with low motility are cultured in this fluid, they transforms into sperm with high fertility [32]. It is also possible to keep matured sperm refrigerated in this fluid for about 1 month without losing high fertility [32], facilitating the systematic production of sperm for artificial insemination.

It has been shown that carrying out artificial insemination quickly after ovulation is vital to improving fertilization performance. This is because as the time between ovulation and fertilization grows longer, the rates of fertilization and hatching fall dramatically [33]. Also, spawning induction technology, whereby male and female parental eels with maturity induced by administering hormones are housed in a spawning tank to promote natural spawning within the tank, has advanced greatly in recent years. Spawning has been successfully induced to an extremely high probability by controlling the water temperature, and the obtained egg fertilization and hatching performance also exhibited better results than artificial insemination [34].

In connection with improving egg quality, the NRIA group has demonstrated, from the component analysis of rarely obtained good-quality eggs, the importance of a high vitamin C content and a suitable vitamin E content [35], and has indicated that the quality of eggs can be improved through nutritional enrichment. Finally, reducing the water temperature from the previous 20–15 °C for maturity inducement makes it easier to set the timing of the final oocyte maturation inducement, and increases the probability of obtaining good-quality eggs.

Development of larval rearing technology for metamorphosis to glass eels

In the research by Yamamoto and Yamauchi [5], artificially inseminated eggs hatched at 23 °C, but the suitable water temperature for hatching was not studied in detail. Moreover, the hatching temperature and larval habitat depth in the wild had not been clarified at that time either. Therefore, in the mid-1990s, when reliable artificial insemination became possible at NRIA, detailed studies were conducted to investigate the optimal temperature for hatching, as well as the optimal temperature until the start of the feeding stage, the reaction of larvae to the light, and other larval rearing conditions [36]. To determine the optimal hatching temperature, fertilized eggs were cultured in seawater at several temperatures between 16 and 30 °C, in increments of 3 °C. Hatching was observed in the 19–28 °C range, while at 19 °C, the hatching rate was low and most of the hatched larvae were deformed in shape. Normal larvae were obtained with a high hatching rate within a range of 25 ± 3 °C [36]. But when mass fertilization was attempted, the water quality quickly deteriorated due to the decomposition of dead eggs at high temperatures, and hatching often became unlikely.

Based on these results, when research on larval rearing first started at NRIA, a water temperature near the bottom end of the range for normal hatching was used as the hatching temperature for larvae used in rearing tests. At water temperatures of 22–23 °C, preferable hatching was achieved in the research; larvae hatched after 35–40 h and opened their mouths 3–4 days after hatching. Their eyes turned black after 6 days, and they showed pronounced negative phototaxis from the ninth day after hatching [36]. Meanwhile, from the sixth day after hatching, when the eyes became functional and the mouth faced forwards, the development of the pancreas was observed, digestive enzymes began to be secreted, and it was confirmed that the mechanisms of eating, digesting and absorbing feed were complete [37, 38]. Recent research on the rearing environment of eggs and hatched larvae has shown that there is little occurrence of deformity at higher temperatures (24–25 °C) and higher salinity (34–35 ‰) compared to in the conventional rearing environment [39, 40]. This condition conformed very closely to the habitat environment of preleptocephali collected in spawning ground surveys [20].

Even after 1973, when Yamamoto and Yamauchi [5] succeeded in artificially hatching of larvae for the first time in the world, there were no clear records on feed-based larval rearing tests until the end of the 1980s; larvae could only be kept alive up to a total length of around 7 mm for 2 weeks after hatching, when the supply of nutrients from the egg became exhausted. Because of the lack of knowledge about suitable feed for hatchlings, feed-based rearing had never been achieved. Even at NRIA, where larvae could be obtained repeatedly in numbers far exceeding those ever obtained in previous research studies, the first-made attempt was feeding rotifers, which is a common initial feed, indispensable in seedling production of most marine fish species. In their trials, larvae at various stages of growth were repeatedly fed on rotifers, and as a result, in 1994, 13-day-old larvae were confirmed to ingest rotifers for the first time [41].

Despite repeated feeding tests, however, no feeding effects, such as growth after complete absorption of the yolk and prolongation of the survival period, appeared when larvae were reared on rotifers. Candidate feeds were therefore re-assessed from a wide range of possibilities, and the larvae were found to feed voraciously on low-temperature dried powdered shark eggs sold commercially for nutritional enrichment of live feed organisms (product name Aquaran, BASF Japan Ltd., Tokyo). However, powdered shark eggs caused a rapid deterioration of the water quality in the rearing tank. In order to solve this problem, a rearing method was devised whereby the remainder of

larvae feed was promptly washed away at a certain point after every feeding. This not only improved the water renewal rate, but also ensured a long-term rearing facility, with hygienic rearing tanks replaced every night [42].

With this rearing method, for the first time in the world, it was confirmed that eel larvae were able to digest and absorb the feed they ate, and this discovery extended their survival period and growth. However, when fed with powdered shark eggs only, larvae could only grow for about 1 month after hatching, and reached a total length of merely 10 mm. They did not develop into the deep-bodied willow-leaf shape that is a morphological characteristic usually found in wild leptocephali [42].

Therefore, feed was reassessed, with an aim of discovering what element was missing in shark eggs as the feed for larvae. This led to the development of a new feed that was based on powdered shark eggs, with an addition of oligopeptides (proteins decomposed by enzymes) that would be easily absorbed even by larvae that had not yet fully developed digestive functions. Vitamins and minerals that were fortified and suspended in krill extract fluid were also added as feed ingredients. In addition, the rearing water temperature was slightly reduced to around 21.5 °C, and the feeding frequency was increased from four to five times a day.

As a result, in 1998–1999, healthy growth continued to the 20th day after hatching, at which point growth had stagnated in the previous rearing method. After 30 days, larvae reached an average length of 10 mm; after 50 days the length was more than 15 mm, and on the 100th day, it was more than 20 mm. Moreover, from about the time when the total length exceeded 10 mm, the body depth relative to the body length started to increase. After this, other morphological changes (the development of teeth and fins, an increase in the number of preanal myomeres, etc.) were also observed. A survival record of more than 250 days was achieved, with larger individuals growing to total lengths of more than 30 mm [9]. However, not only was the growth rate slower than larvae in the wild, but the leptocephali thus obtained were weak, and gradually became depleted during the longer rearing period. None of the reared leptocephali reached a length of 50–60 mm, the size when metamorphosis into a glass eel is supposed to occur.

To encourage the healthy growth of larvae to the size when metamorphosis starts, it was thought essential to maintain water quality and keep the inside walls of the tank clean. To this end, a shorter feeding time and careful cleanup of the tank's inside walls after every feeding time were promoted, and illumination was increased from the normal level especially during the feeding time. As a result, larval feeding activity intensified, which successfully encouraged the larvae's energetic consumption of feed in a short duration of time.

The ingredients of the feed were also rigorously scrutinized. The soy peptides conventionally used as feed additives included a phosphorus compound called phytic acid, and it became clear that this could inhibit the absorption of minerals and proteins. In a joint research with Fuji Oil Co. Ltd., soy peptides with a less phytic acid effect were developed by enzyme treatment and tested using a trial product. In another joint research with Nippon Suisan Kaisha, Ltd., the additive effect of krill hydrolysate that was manufactured in a test with an aim of efficient use of effective components of krill was also investigated. Meanwhile, frozen shark eggs before the drying process were used in place of the manufacture of low-temperature dried powdered shark eggs (the principal ingredient of conventional feed).

Various types of feed were prepared with different proportions of these ingredients and vitamins, as well as other ingredients. Using these, feeding tests were carried out repeatedly over 10 days, 20 days, and even during longer periods after the start of feeding. As a result, artificially hatched eel larvae grew more favorably than before, until just over 100 days after hatching, when they exceeded a total length of 30 mm, eventually growing to the target length of 50–60 mm in 200–300 days. Then, in May 2002, one of the leptocephali that had reached this size was found to have a slightly longer tail. On closer inspection, the position of the anus was found to have shifted forward, and the body depth was also slightly reduced. Within a few days, this individual sank to the bottom of the tank and lay still with increasing frequency, changing its appearance to the cylindrical shape of a glass eel [12]. A developmental growth series of a larva reared in the laboratory, from newly hatched larva to the maximum size at the end of the leptocephalus stage, to metamorphic changes into the glass eel stage, are indicated in Fig. 1.

Since then, at NRIA, the metamorphosis process has been observed in detail for more than 200 specimens, and it is now known that the main changes accompanying metamorphosis are a forward shift of the anus and the base of the dorsal fin, a reduction in body depth, loss of mucopolysaccharide tissue with the development of muscle, development of gills, appearance of erythrocytes (red blood cells), loss of needle-like teeth, decrease in eye diameter, and the appearance of black pigments [11, 12]. These changes accompanying metamorphosis continued to occur at around 20 days at a water temperature of 21.5 °C, but were complete in only around 10 days at 25 °C, proving experimentally that the progression of metamorphosis depends to a large extent on temperature.

In a research project that started in 2005 and was commissioned by the Agriculture, Forestry and Fisheries Research Council (AFFRC), nearly all the researchers connected with eel research in Japan, including those at the FRA and prefectural fisheries research institutes,

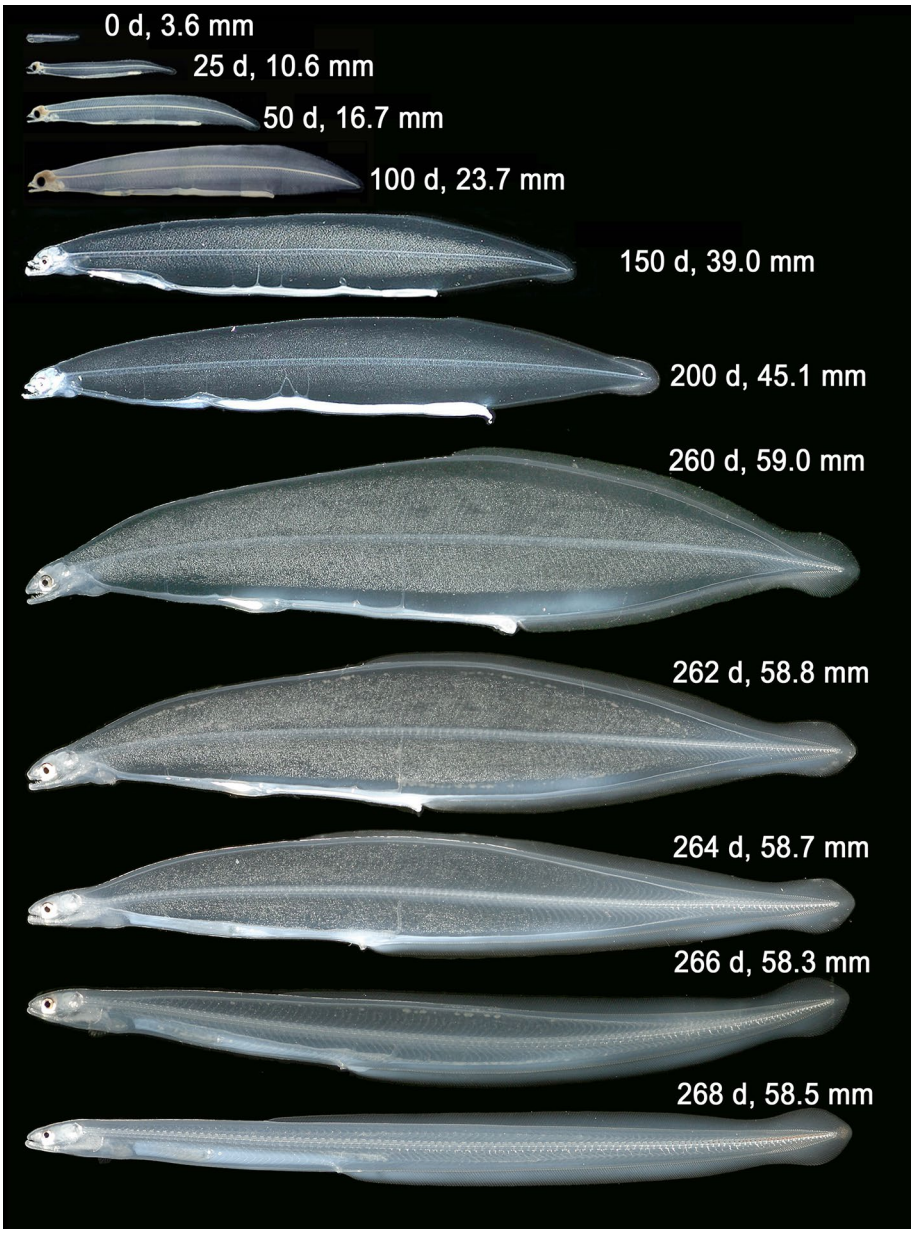

Fig. 1 A developmental growth series of an *Anguilla japonica* larva reared in the laboratory, from a newly hatched larva (preleptocephalus, 3.6 mm in total length, 0 days old) to the maximum size at the end of the leptocephalus stage (59.0 mm, 260 days old), to the glass eel stage (58.5 mm, 268 days old). The body size of the leptocephali diminished slightly during the process of metamorphosis. Specimens older than 260 days are the same individual. Reprinted with kind permission from Springer Science + Business Media: Marine Biology, Positive buoyancy in eel leptocephali: an adaptation for life in the ocean surface layer. 156, 2009, 835–846. Tsukamoto et al. Fig. 2. © 2009, Springer-Verlag. Original photographs provided by Hideki Tanaka of the National Research Institute of Aquaculture [43]

universities, etc., have joined forces in tackling challenges such as improving cultivation and maturation technology for parental eels, developing feed for larvae, and optimizing the rearing environment. The target is a tenfold increase in the survival rate of larvae until the 100th day after hatching under the artificial rearing environment. Viewing the various outcomes collectively obtained from this project in terms of rearing, the survival rate from the start of feeding to the 100th day after hatching, which was around 0.2 % before the project started, increased greatly to around 20 % by 2011. The subsequent survival rate up to the glass eel stage, although still somewhat unstable, has reached 1–5 %. Currently, research is being continued with the target of developing a stable production technology for glass eels.

Attainment of full-life cycle culture and future prospects

Taking this one step further, FRA has continued to nurture artificially produced eels at the NRIA's Nansei Main Station and Shibushi Laboratory, with the aim of raising them to parental eels and achieving full-life cycle culture. At the end of 2009, it was confirmed that maturation induction was possible in both the male and female eels that had been reared to this stage. From the beginning of 2010, therefore, a hormonal treatment for maturation induction was started with an aim of achieving full-life cycle culture. However, some eels were found to be suffering from a change in their physical conditions as their maturity advanced, and on that

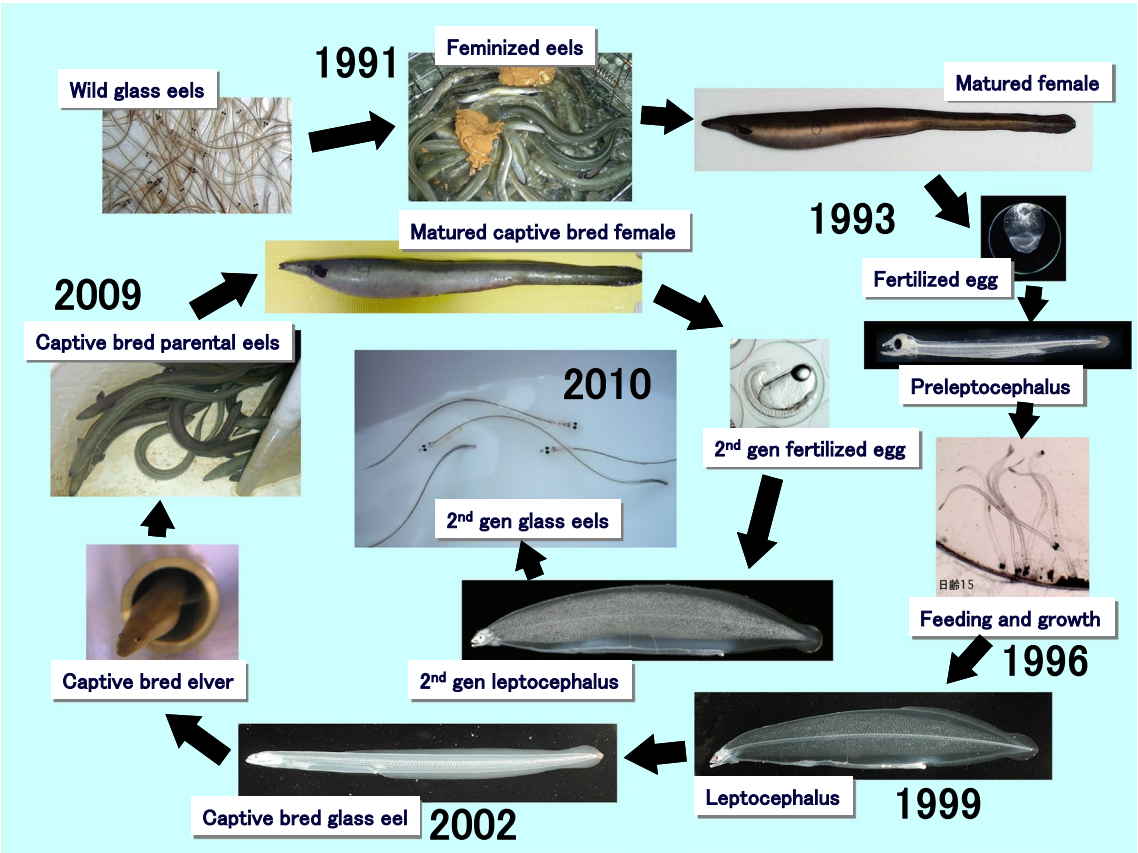

Fig. 2 Progression of research on eels until the attainment of full-life cycle culture. *Numerals* show the years when each stage was reached

point, it seemed doubtful whether the full-life cycle culture could be achieved. Nevertheless, the Shibushi Laboratory obtained a total of more than two million eggs from nine females after March 26th, showing good performance in both fertilization and hatching rates, and numerous second generation larvae were born (Imaizumi et al., private communication). At the Nansei Main Station, fertilized eggs were also obtained from two females despite some difficult conditions, and hatching was confirmed, albeit in a small volume.

The artificially produced second generation eel larvae obtained at the Shibushi Laboratory have exhibited unprecedented good survival rates and growth, thanks to dedicated rearing management by laboratory staff members, and from the summer of 2010 onwards, the larvae successively metamorphosed into glass eels [44]. Some of the larvae were sent to the Nansei Main Station soon after hatching, and these are continuing to grow favorably there as well. Although it needs to be verified in the future, this good rearing outcome was probably because the larvae were artificially produced second generation, and could easily adapt themselves to the rearing environment. Or it could be due to progress and improvements in the rearing technology. In any case, the larvae were confirmed to be healthy, with a capability for favorable growth. As a result, a "full-life cycle culture", whereby generations of eels are accumulated in the rearing environment without depending on any supply from wild resources, was attained for the first time in the world [45].

In Fig. 2, the author summarizes the progression of research on eels over some 20 years, from the start of involvement in the research of artificial seedling production up to the achievement in full-life cycle culture. Looking back at the past, it took just over 3 years from the fully fledged start of research at NRIA in 1993 until the discovery of shark eggs as a key ingredient of the larval feed. At the time, it felt longer; this was a most agonizing period in which, although feeding on rotifers was confirmed, no growth in larvae was seen at all and the prospects seemed hopeless. But despite a number of obstacles still arising one after another, many researchers who assembled for the eel project finally succeeded in overcoming the problems through their concerted efforts.

With the attainment of full-life cycle culture in 2010, eel farming without dependence on fry supplies from wild resources has become theoretically possible. However, in

order to realize an actual use in the commercial industry and to provide a stable supply of eels to dinner tables, it is essential to develop a technology for mass production of glass eels. To this end, it will be indispensable to secure a stable supply of good-quality larvae, and to create innovations in feed and rearing methods to rear larvae in bulk.

As recent innovative technology for rearing eel larvae, rearing in low-salinity environments that could not occur in the wild and inducing metamorphosis through starvation may be cited [46–48]. Until now, leptocephali growth under rearing conditions has been slower than growth in the wild, and the period until the onset of metamorphosis has been protracted. This causes problems in terms of a declining survival rate and the rising cost of feed in that period. To remedy these problems, Okamura et al. [46] proved that higher survival and growth rates could be promoted by rearing artificially hatched eel larvae in 50 % diluted seawater (17.5 psu) compared to rearing larvae in the 100 % seawater environment. Wild eels inhabit the open seas during the larval phase, and it is inconceivable that they could be exposed to low salinity environments there. Nevertheless, this improvement in rearing performance is thought to have resulted from rearing in a low salinity environment approximating the osmotic pressure of body fluids, thereby enabling eels to conserve the energy necessary for osmoregulation.

Another occasional problem was that, in the rearing environment, metamorphosis could not start even after eels reached a total length of 60 mm or more, which is beyond the size at which metamorphosis occurs in the wild; the leptocephalus phase thus lasted more than a year. Although attempts were made to induce metamorphosis by methods such as adjusting the rearing water temperature, no clear effects were obtained. To address this problem, Okamura et al. [48] closely examined the impact of starvation on metamorphosis, and found that the onset of metamorphosis in leptocephali with a total length of 55 mm or more can be significantly induced through starvation. In the artificial production of glass eels, this technology is very valuable in that it can induce not only one individual's metamorphosis quickly, but can also synchronize other previously untapped sporadic metamorphoses, which enables significant numbers of glass eels to be obtained at the same time.

If mass production of glass eels were to be made possible and some of the fry for aquaculture could be furnished using captive-bred eels in the future, it would be possible not only to protect wild eel resources, but also, to a certain extent, to stabilize the supply of seedlings (which used to be unstable as it relied on the size of wild fry catches). Moreover, by accumulating generations of captive bred eels, it is also expected that "full-life-cycle-culture-branded" eels that are easy to rear, delicious, safe and reassuring in supply can be produced. Therefore, further research and development on technologies aimed at the stable mass production of artificial eel seedlings is expected.

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