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# Temperature Adaptation of the Japanese Eel (*Anguilla japonica*) in its Early Stages

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**Su-Lean Chang, Guang-Hsiung Kou and I Chiu Liao (2004)** Temperature adaptation of the Japanese eel (*Anguilla japonica*) in its early stages. *Zoological Studies* **43**(3): 571-579. The biology and ecology of Japanese eel (*Anguilla japonica*) in the early developmental stages are still not clear. This study was conducted to determine the optimum water temperature for incubation of embryos and yolk-sac larvae of this eel. Results show that both embryos and yolk-sac larvae are able to adapt to wide ranges of water temperatures. The embryos at the morula, gastrula, and C-shaped stages initially incubated at 23°C were able to adapt to temperatures of 18~28°C. However, the hatching rate was significantly lower when morula and gastrula stages were transferred to 18°C. The survival rate at the eye-pigmented stage was highest at 26°C for all 3 embryonic stages. Moreover, C-shaped embryos were able to tolerate a higher temperature (30°C) if acclimated to 26°C prior to the trial. One-day-old yolk-sac larvae, on the other hand, were able to adapt to temperatures of 3~32°C. The highest survival rates at the eye-pigmented stage were observed at 26~30°C. From the above results and considering other technicalities of spawning and incubation, it is suggested that water temperatures be regulated in the range of 24~26°C for incubation of Japanese eel embryos, and 26~28°C for incubation of yolk-sac larvae. Based on temperature adaptation and good buoyancy of eel embryos, we speculated that eel eggs may float up to the warm water layer of the sea (> 24°C) which is above the putative spawning water layer (100 m in depth). On the other hand, hatched yolk-sac larvae may sink to the colder water layer and dim environment based on their tolerance of low temperatures, subsidence attributes, and negative phototactic behavior during the eye-pigmented stage. http://www.sinica.edu.tw/zool/zoolstud/43.3/571.pdf

**Key words:** *Anguilla japonica*, Embryo, Yolk-sac larvae, Water temperature.

Eels are well known for their mysterious reproductive biology and unique morphology in the larval stage. Much information has been reported with regard to their spawning grounds (Schmidt 1922, Kleckner et al. 1983, Jellyman 1987, Tsukamoto 1992, Liao et al. 1996 1999, Liao 2001) and larval dispersal in the open ocean (Boëtius 1985, Ozawa et al. 1992, Otake et al. 1998). However, little is known about the spawning migrating routes of the spawners, the exact water layers used at the spawning grounds, and to what water layer the eggs drift after spawning. This is due to a lack of spawners and fertilized eggs being collected from the sea (Tsukamoto 2001). Eels are widely distributed in different latitudes and are capable of tolerating a wide range of water temperatures. In comparison with the European eel (*Anguilla anguilla*), the Japanese eel (*A. japonica*) is reported to be more tolerant of higher water temperatures (Matsui 1972, Sadler 1979).

Optimum water temperatures for spawning and early larval development of Japanese eel are still not exactly known. Japanese eel are commonly induced to ovulate and spawn at a temperature of 22~23°C (Ohta et al. 1996, Liao and Chang 1999). Consequently, the temperature for incubating fertilized eggs of Japanese eel is in the same range (Yamamoto and Yamauchi 1974, Yamauchi et al. 1976, Tanaka et al. 1995).

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However, no report is available on a suitable temperature range for incubation of fertilized eggs and yolk-sac larvae of Japanese eel. In addition, the temperature for larval rearing of Japanese eel varied with different researchers: 23~26°C for up to 19-d-old larvae (Wang et al. 1980); 15~19.5°C for up to 25-d-old larvae (Yu et al. 1993); 22~23°C for up to 18-d-old larvae (Tanaka et al. 1995); and 21.5~23°C for up to 29-d-old larvae (Tanaka et al. 2000). Tanaka (1999) found that Japanese eel larvae feed more on rotifers at higher temperatures (19~28°C). In addition to the problem of suitable larval feed, the occurrence of mortality during larval rearing might also be attributed to unsuitable water temperatures.

The Japanese eel supposedly spawns at a water depth of 100 m (Tsukamoto 1992), at a temperature of around 24°C (Kajihara et al. 1988). In the laboratory, eel eggs are reported to have good buoyancy but the yolk-sac larvae exhibit vertical subsidence during yolk-sac absorption. Differences in temperature adaptations at different stages during early development are possible. Thus, different developmental stages at early (morula), middle (gastrula), and late embryonic stages (C-shaped embryos) as well as the early larval stage were chosen in order to determine their temperature adaptation ranges. In addition, juvenile and adult eels can tolerate extremely low water temperatures (1~3°C) even for extended periods (Matsui 1972, Tongiorgi et al. 1986). Little is known, however, about the tolerance of early stages to low water temperatures.

This study was conducted to investigate the effects of water temperature on the different embryonic stages of Japanese eel and to determine the tolerance of yolk-sac larvae to low and high water temperature limits. Optimum temperatures for incubation of both eggs and larvae were determined.

## MATERIALS AND METHODS

### Egg source

Fertilized eggs were obtained by induced spawning of pond-reared Japanese eel based on the protocol reported by Liao and Chang (1999 2001). Eggs were incubated in a cylindrical holding net (70 cm in diameter, 60 cm deep) with mild aeration and slow water flow. The holding net was suspended in a 2 $m^3$ concrete tank (2x1x1 m) supplied with flow-through seawater (30~33 ppt). The water temperature for incubation of fertilized eggs was controlled at 23 ± 0.5°C.

### Egg development and hatching times at different temperatures

Egg development at 23°C was determined by inspection using a binocular microscope. The onset of hatching time was determined by incubating the fertilized eggs (1 h after fertilization) at water temperatures of 18, 20, 22, 23 (control) 24, 26, and 28°C. About 500 eggs were stocked in a 1 L vessel. Embryos in the vessel were then immersed in temperature-controlled basins (20, 22, 23, 24, and 26°C). At 18 and 28°C, the temperature change was controlled at 1°C/h after 1 h of acclimation at 20 and 26°C, respectively.

### Water temperature adaptation of embryonic stages

Normally developing embryos at the morula (5 h after fertilization), gastrula (15 h, 1/2 epiboly), and C-shaped (24 h, eye vesicles formed) stages were chosen under a binocular microscope on a multi-cavity loading slide using an injection needle, and were separated from malformed embryos. Thirty embryos were collected from each stage using a pipette, and these were transferred into a polyester rectangular vessel containing 1 L of seawater. Embryos in the vessel were then immersed into temperature-controlled basins (at 18, 20, 22, 24, 26, and 28°C) with 4 replicates per treatment (temperature levels were adjusted as described above). Hatching times varied between 6 and 55 h depending on the temperature levels and embryonic stages tested. Light aeration (30 ml/min) was provided to keep the eggs afloat, but this was terminated after hatching.

Both normal (without a pronounced curvature of the body trunk) and malformed larvae were counted 6~12 h after hatching. Larvae were transferred into another vessel with 1 L of fresh seawater and incubated further at the different temperature treatments (18, 20, 22, 24, 26, and 28°C) until the eye-pigmented stage. No aeration was provided during this interval. The development of yolk-sac larvae to the eye-pigmented stage varied from 4 to 11 d depending on the temperature levels tested. The eye pigment of larvae gradually forms in association with transformation of the swimming behavior, from darting behavior to snake-like swimming. This transformation lasted about 1~2 d depending on water temperature levels. The tim-

ing for counting the number of eye-pigmented larvae was undertaken at the onset of snake-like swimming behavior. At the estimated counting time, the development of larvae was frequently inspected.

**Adaptation to high water temperature**

1. Water temperature increase from the C-shaped embryonic stage

Water temperature for incubation of fertilized eggs was increased gradually from 23 to 26°C before the C-shaped stage. After incubation for 24 h, C-shaped embryos with normal development were selected using the binocular microscope. Thirty embryos were transferred to a vessel containing 1 L of seawater. Vessels with embryos were immersed in 4 temperature-controlled basins at 26°C. The water temperature treatments of the 3 basins were regulated to respectively increase 1°C/h from 26 to 28, 30, and 32°C. One basin was maintained at 26°C (as the control). Every water temperature treatment had 4 replicates. Mild aeration (30 ml/min) was provided in every vessel, but this was terminated after hatching. One-day-old normal larvae were transferred into another vessel with fresh seawater. Larvae were incubated at the same adapted water temperature with no aeration until the eye-pigmented stage, and then counted.

2. Water temperature increase from 1-d-old yolk-sac larvae

With the same procedure as described above, 30 C-shaped embryos in a vessel were incubated at 26°C. After hatching, 1-d-old larvae were transferred into another vessel with fresh seawater. The water temperature was then regulated to increase 2°C/d by transferring the vessels containing larvae directly into 28, 30, and 32°C basins on the 1st, 2nd, and 3rd days. Each treatment had 4 replicates, and no aeration was provided. The survival of yolk-sac larvae was counted at the eye-pigmented stage.

**Low water temperature limit of yolk-sac larvae**

Normal 12-h-old larvae were selected at a water temperature of 24°C. Twenty larvae were transferred into each vessel with 1 L of seawater. Vessels were immediately placed into a refrigerator while the larvae were developing to 1 d old. The change in water temperature in the 1st hour was prominent (7~8°C), less prominent at 2~4 h (2~4°C), and then gradually stabilized at 1°C after 4~5 h. Four replicate vessels with larvae were taken out of the refrigerator when the temperature had decreased to 7, 5, and 3°C, respectively, and these were incubated at ambient room temperature without aeration. The survival of larvae was counted at the eye-pigmented stage.

**Data analysis**

All percentage data were normalized by square- or angular-transformation prior to the statistical analysis. The statistical significance of the hatching rate, percentages of normal and malformed larvae, and survival rate of eye-pigmented larvae was tested by analysis of variance (one-way ANOVA), followed by Duncan's multiple-range test. Statistical analyses were performed using the SPSS (vers. 10.0; SPSS Inc., Illinois, USA) statistical software.

## RESULTS

**Egg development and hatching times at different temperatures**

Egg development at 23°C proceeded as follows: 1st cleavage at 68 min, morula at 3 h, blastula at 8 h, gastrulation at 10 h, 1/2 epiboly at 15 h, closing of the blastopore at 20 h, and formation of the eye vesicle at 24 h. Eggs hatched at 48, 40, 36, 34, 28, and 24 h at 20, 22, 23, 24, 26, and 28°C, respectively. It was also observed that embryonic development was completely retarded when fertilized eggs were transferred from 23 to 18°C 1 h after fertilization.

**Water temperature adaptation of embryonic stages**

Hatching rates (72.5%~87.5%) and percentages of normal larvae (58.3%~75.8%) were higher when morula embryos were incubated at temperatures of 20~28°C (Fig. 1a, b). On the other hand, the hatching rate (23.3%) and percentage of normal larvae (8.3%) were significantly lower ($p < 0.01$) when embryos were incubated at 18°C compared to those at 20~28°C. Numbers of malformed larvae were high at 18 (15.0%) and 20°C (14.2%) compared to the other treatments (4.2%~11.7%) (Fig. 1c). Survival at the eye-pigmented stage was lower at 18 (5.0%) and 20°C (15.0%) (Fig. 1d). Similar results were obtained

for the gastrula stage when transferred to various temperature levels (Fig. 2a-d).

For C-shaped embryos, hatching rates (80.8%~92.5%) and percentages of normal larvae (74.2%~86.7%) were high at all temperature levels tested (18-28°C) (Fig. 3a, b). No significant differences were observed among the treatments ($p > 0.05$). Meanwhile, the percentage of malformed larvae (14.2%) was highest at 28°C (Fig. 3c), while survival at the eye-pigmented stage (50.8%)

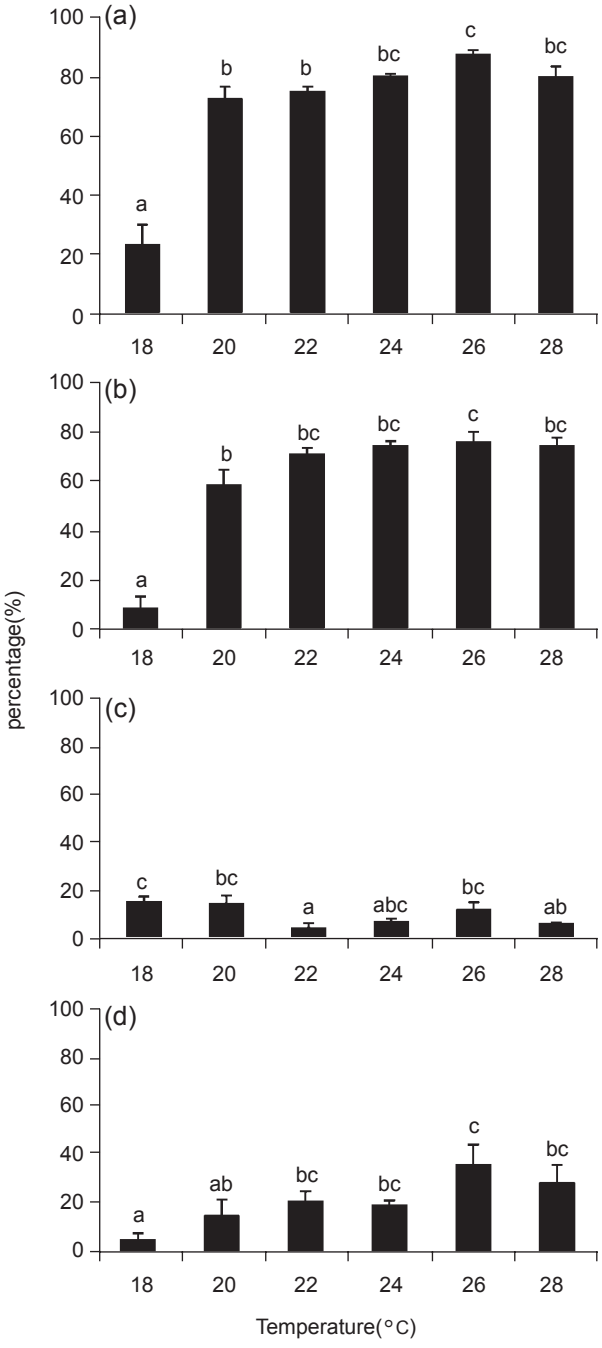


**Fig. 1.** Hatching and survival rates of normal, malformed, and eye-pigmented larvae after incubation of morula embryos and yolk-sac larvae at different water temperatures. (a) Hatching rate, (b) normal larvae, (c) malformed larvae, (d) eye-pigmented larvae. Bars (±S.E.) with different superscripts denote a significant difference at $p < 0.05$.

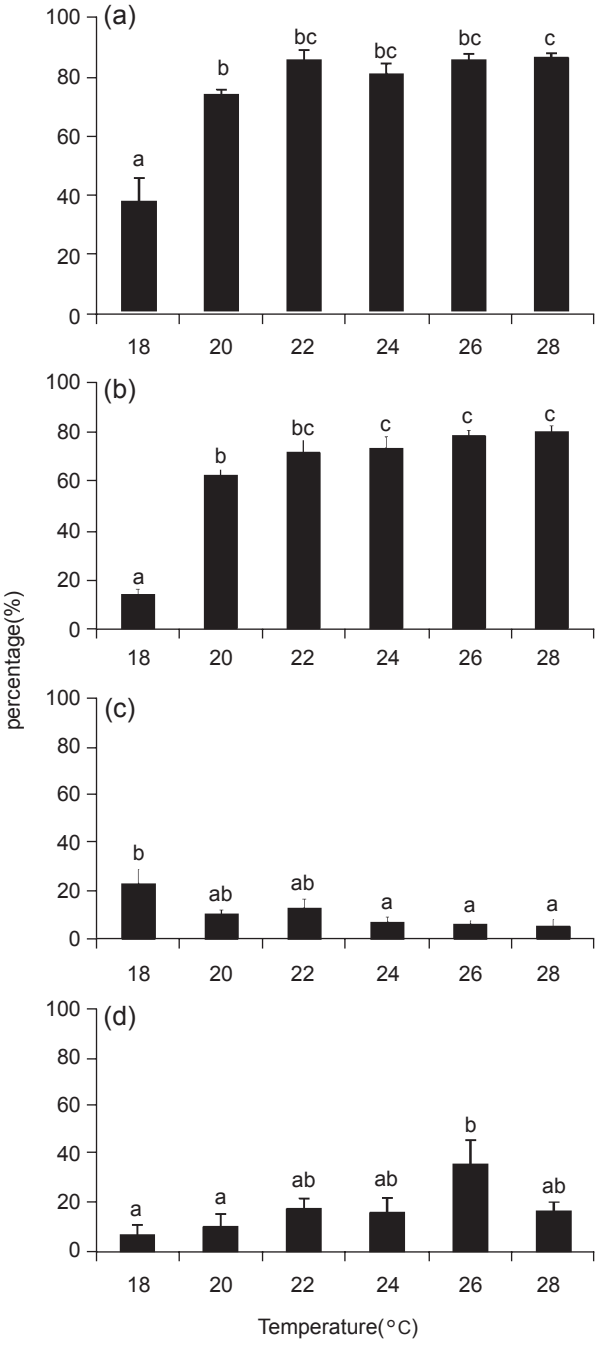


**Fig. 2.** Hatching and survival rates of normal, malformed, and eye-pigmented larvae after incubation of gastrula embryos and yolk-sac larvae at different water temperatures. (a) Hatching rate, (b) normal larvae, (c) malformed larvae, (d) eye-pigmented larvae. Bars (±S.E.) with different superscripts denote a significant difference at $p < 0.05$.

was highest at 26°C (Fig. 3d). Newly hatched larvae required 4 d at 28°C, 4~5 d at 26°C, 7~8 d at 22°C, and 10~11 d at 20°C to reach the eye-pigmented stage.

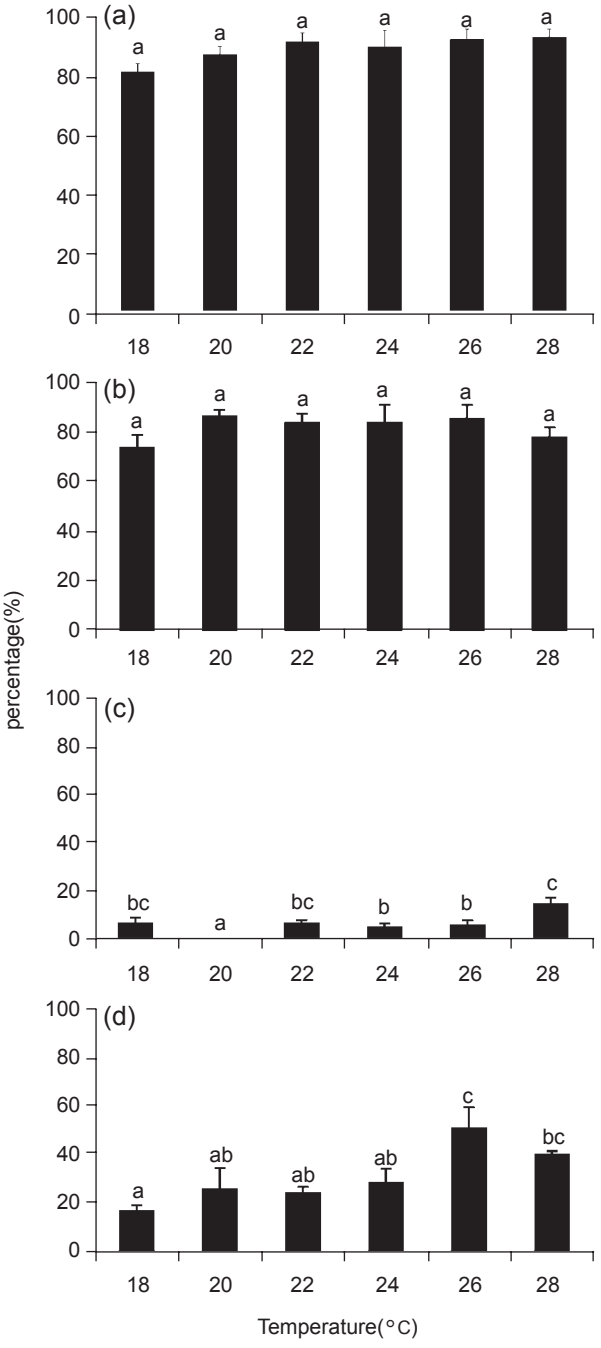


**Fig. 3.** Hatching and survival rates of normal, malformed, and eye-pigmented larvae after incubation of C-shaped embryos and yolk-sac larvae at different water temperatures. (a) Hatching rate, (b) normal larvae, (c) malformed larvae, (d) eye-pigmented larvae. Bars (±S.E.) with different superscripts denote a significant difference at $p < 0.05$.

## Adaptation to high water temperature

Percentages of normal larvae were significantly higher ($p < 0.01$) at 26 (50.8%), 28 (59.2%), and 30°C (46.7%) than that at 32°C (17.5%) after transferring C-shaped embryos from the acclimation temperature of 26°C (Fig. 4). Survival of eye-pigmented larvae, on the other hand, was significant lower ($p < 0.01$) at 30°C (8.3%) compared to that at 26 (41.7%) and 28°C (30.8%); no larvae survived at 32°C. When yolk-sac larvae were transferred from the acclimation temperature of 26°C to the same test temperature levels, survival rates of eye-pigmented larvae were higher at 26 (43.3%), 28 (38.3%), and 30°C (41.7%) (Fig. 5). Moreover, surviving larvae were also observed at 32°C (17.5%) although at a significantly lower per-

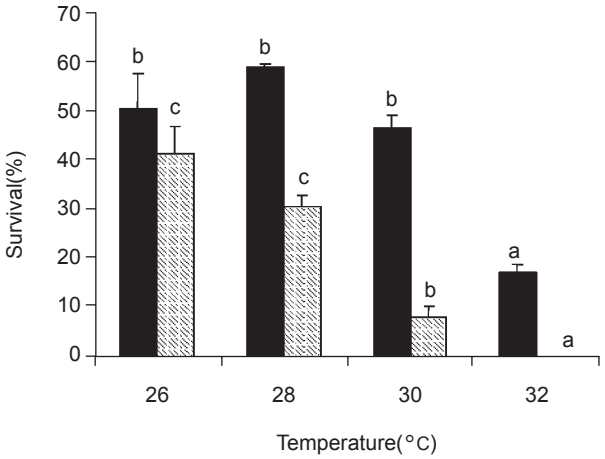


**Fig. 4.** Survival rates of normal and eye-pigmented larvae after transfer of C-shaped embryos from 26 to 28, 30, and 32°C. Bars (±S.E.) with different superscripts denote a significant difference at $p < 0.01$.

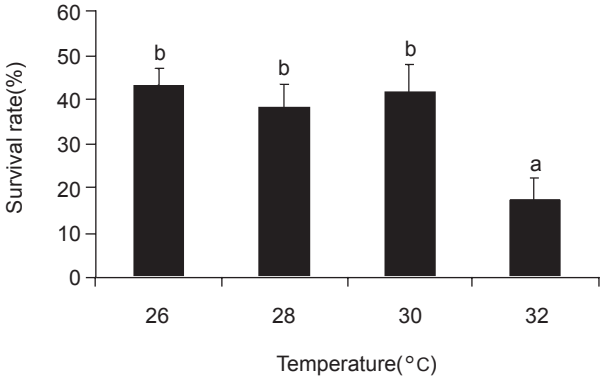


**Fig. 5.** Survival rates of eye-pigmented larvae after transfer of 1-d-old yolk-sac larvae from 26 to 28, 30, and 32°C. Bars (±S.E.) with different superscripts denote a significant difference at $p < 0.01$.

centage ($p < 0.01$) compared to those at 26, 28, and 30°C.

### Low water temperature limit of yolk-sac larvae

The survival rate of eye-pigmented larvae (42.5%) was significantly higher ($p < 0.05$) when yolk-sac larvae were transferred to a refrigerator until 7°C was reached, compared to those larvae exposed to lower temperatures of 5 and 3°C (16.3% at both temperatures; Fig. 6). The survival rate after exposure to 7°C (42.5%) was comparable to the control (40%) treatment (yolk-sac larvae maintained at 24°C).

Compared with the embryonic stage, yolk-sac larvae showed tolerance to an extremely low water temperature (3°C) during a temporary challenge. However, it was observed that the activity of the larvae was markedly reduced at water temperatures of 15°C and below in a similar trial. The distances moved decreased from 5~7-fold of the total length at 22°C to 1~2-fold at 12°C, while larvae exhibited no activity at 9°C.

## DISCUSSION

Induced maturation of eels is stimulated by repeated hormonal treatment for more than 2~3 mo (Ohta et al. 1996, Liao and Chang 1999). The quality of eel eggs, on the other hand, is markedly affected by the quality of spawners and the dosage and injection interval of hormones. In addition, low temperatures also often lead to hatching retardation in some embryos. Hatchlings obtained from a retarded hatching process show different degrees of body curvature by visual observation, but some of them can still develop to the eye-pigmented larval stage. In some cases, however, it is difficult to differentiate between normal and malformed larvae, especially among newly hatched larvae. This is why, in this study, the number of hatched larvae was determined 6~12 h after the onset of hatching depending on the temperature level tested. The temperature effect on Japanese eel in the early stage was extended to evaluate the survival of eye-pigmented larvae in order to assess any detrimental effect during the entire incubation period.

The time used in regulating the change in temperature, as well as the duration of exposure to the desired temperature levels, might affect the egg hatching rate and larval survival. In this study, changes in temperature were gradually regulated so as not to exceed 3°C/h during the incubation of embryos. The hatching rate and percentage of normal larvae of morula and gastrula embryos were lower after incubation at 18°C than at 20~28°C. At the C-shaped stage, a high hatching rate was obtained at 18°C, which was comparable to the other temperature levels tested. Moreover, successful hatching was also observed at higher temperatures (30~32°C) after acclimation to 26°C prior to the trial, but with a lower survival rate of normal larvae. The hatching process of fish eggs requires the hatching enzyme chorionase, which hydrolyzes the inner layers of the zona radiata of the eggs and facilitates the rupture of the chorion by the caudal peduncle (Yamagami 1988). Enzyme activity is confined within a certain temperature range. Embryonic development was obviously prolonged at 18°C with the tail having already developed such that it crossed over the head leading to hatching failure or a high percentage of larvae with spinal curvature after hatching. The activity of the enzyme might be inhibited at this temperature.

From the results obtained in this study, it can be speculated that the Japanese eel might not spawn in the cold, deep-water layer. Under laboratory conditions, eel spawners that attained final maturation usually hid in the tube shelter at 20°C, but they swam out of the tube and performed mating chases when the water temperature was increased to 21.5°C (Satoh et al. 1992). Sato et al. (1998) found that the gonad development of hormone-treated female Japanese eels was far

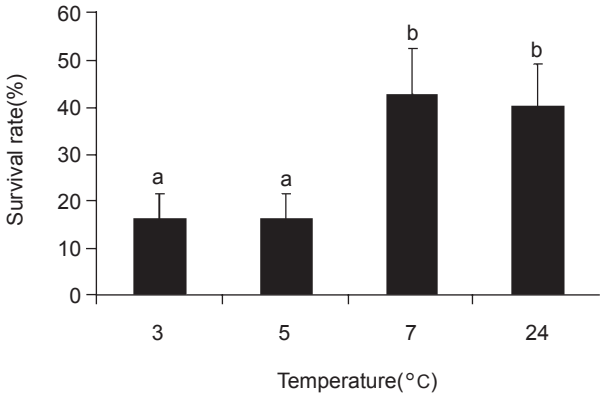


**Fig. 6.** Survival rates of eye-pigmented larvae after transfer of 1-d-old yolk-sac larvae from 24°C to a refrigerator. Vessels containing larvae were taken out of the refrigerator when the water temperature had reached 7, 5, and 3°C, and were then placed back at the ambient temperature of 24°C until the eye pigment had formed. Bars (±S.E.) with different superscripts denote a significant difference at $p < 0.05$.

better at 20°C (gonadosomatic index, GSI = 32) than at 10°C (GSI = 3.4). Liao and Chang (1999) found that aged Japanese eels spawned more frequently without administration of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) at a water temperature of 23°C than at 22°C. Based on these results, it can be speculated that eel spawners might swim up to a warmer water layer for spawning.

The range of water temperatures used in this study for the incubation of eel embryos (18~28°C) is similar to the adopted water temperature range for the incubation of embryos of typical warm-water marine fishes such as the orange-spotted grouper (*Epinephelus coioides*) (22.1~31.0°C) (Kawahara et al. 1997) and blue fin tuna (*Thunnus thynnus*) (21.2~29.8°C) (Miyashita et al. 2000). On the other hand, embryos of black sea bream (*Acanthopagrus schlegeli*), a temperate marine finfish, hatched even at 15, 13, 13, and 11°C after transferring the morula, gastrula, C-shaped, and pre-hatching embryos, respectively, from 23°C (Chu et al. 1996). Tolerance to a change in the low water temperature range of black sea bream is 8°C in the morula stage and 10°C in C-shaped embryos. It is markedly stronger than the Japanese eel which is only about 5°C (23 to 18 °C) in the morula stage.

Based on the results of hatching rates in this study with the adaptation of Japanese eel embryos to different water temperatures, the optimum range for incubation was between 20 and 28°C. However, considering the different hatching periods at different water temperatures, together with the practicality of regulating temperatures of the spawning water (22~23°C), it is then suggested that the water temperature for egg incubation of Japanese eel be regulated in the range of 24~26°C.

In the putative spawning grounds of Japanese eel in the Western Pacific, water temperatures are about 10, 17, 20, and 24°C at the depths of 500, 300, 200, and 100 m, respectively (Kajihara et al. 1988). Since eel eggs possess good buoyancy at 30 ppt under laboratory conditions, it is then speculated that eel eggs might float up to a warm water layer of the sea (> 24°C) which is above the putative spawning water layer (100 m in depth).

In the laboratory, yolk-sac larvae were observed to gradually sink to the bottom during the process of yolk-sac absorption and lie there until the late stage when they began snake-like swimming. Larvae lying on the bottom are either prone to predation or easily disturbed by bottom-dwelling organisms. Incubating yolk-sac larvae at a higher water temperature in their adapted water temperature range can shorten the incubation period to the 1st feeding, thus reducing the risk of yolk-sac larvae staying on the bottom. In this study, yolk-sac larvae of Japanese eel were able to adapt to a wide range of water temperatures (18~30°C) and were even able to survive at 32°C, although at a significantly lower percentage. Moreover, temporary challenge by low temperatures (3~5°C) also showed the tolerance of larvae to a cold environment.

Based on these results, and considering both the stocking period and the suggested water temperature during egg incubation (24~26°C), the water temperature range for stocking yolk-sac larvae of Japanese eel is suggested to be regulated at 26~28°C.

The findings on the adaptation of Japanese eel embryos to low water temperatures, although not obviously strong, have ecological significance in the subsidence behavior of yolk-sac larvae for inhabiting the deeper sea starting from the feeding stage. The oil droplet of yolk-sac larvae is located at the tip of the yolk sac in front of the head, causing them to be suspended in the water column in a vertical posture with the head in the upper position. It is estimated that the period of subsidence until the feeding stage is about 5~7 d at water temperatures of 22~26°C (Liao and Chang 2001). The euphotic zone extends deeper in the clear waters of the oceanic zone, perhaps down to 100~200 m (Odum 1971). The larvae might, therefore, sink to the dim environment because of their negative phototactic behavior during the eye-pigmented stage. It is also speculated that the larvae do not live in a completely dark environment based on their well-developed pigmented eye (Tanaka, 1999), unless larvae depend on bioluminescence for feeding. Thus, the transparent body, vertical posture during subsidence, tolerance to low water temperatures by yolk-sac larvae, and adaptation to the dim environment from the eye-pigmented stage might be innate strategies of eel larvae in avoiding predators and utilizing certain food sources in the lower water layer.

## CONCLUSIONS

Embryos and larvae of Japanese eel can adapt to wide temperature ranges. From the results obtained in this study and considering the

technicalities involved in egg incubation and larval rearing protocols, optimum temperatures for egg incubation (24~26°C) and rearing of yolk-sac larvae (26~28°C) were achieved. The artificial propagation of Japanese eel has not yet been established at this time, even after several decades of research studies in several countries. The findings in this report can serve as a basis for establishing appropriate rearing conditions during the artificial propagation of the Japanese eel. Continued research efforts are therefore recommended towards the goal of the successful mass production of glass eels in captivity.

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