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(Accepted 27 August 2001)

Thermal Effects on Respiratory Activities of Glass Eels at Different Developmental Stages

Abstract

Oxygen consumption rate (OCR) of glass eels in the early (VIA_I) and late (VIB) pigmentation stages was measured in different temperature and salinity regimes to determine the optimal temperature ranges for glass eels. The OCR was determined using an automatic-intermittent flow respirometer (AIFR) under constant darkness condition without food and subjected to a gradual temperature increase (1 °C 24 h^{-1}) for 255.2 to 383.3 h. The OCR of glass eels in the early pigmentation stage (VIA_I) maintained a low value of 0.08 ml O^2 g^{-1} wt h^{-1} by 13 °C, gradually increased as temperature increased from 13 to 20 °C and decreased thereafter. However, the OCR in the late pigmentation stage (VIB) continued to increase as temperature increased up to 25 °C regardless of salinity. These results support that the glass eels are apt to migrate during the cold months, and indicated that their respiratory adaptability to a wider temperature range is increased as the glass eels develop into the late pigmentation stages. The adaptation of the glass eels to the low temperature is considered to be one of the strategies to save energy during migration over continental shelf without feeding after metamorphosis.

Key words: *Anguilla japonica*, Glass eels, Oxygen consumption rate, Thermal effect

Japanese eel *Anguilla japonica* spawns in the salinity front of the North Equatorial Current in the west of the Mariana Islands[1]. The eel larvae, leptocephali, migrate along the warm north equatorial current and Kuroshio Current. The leptocephali are metamorphosed into glass eels around the Kuroshio Current in the south of Taiwan and the Ryukyu Islands[2]. The glass eels migrate over the continental shelf and approach the estuaries of the region. The glass eels are then pigmented and transformed gradually into elvers.

Glass eels begin to occur in Taiwan from November and mainly from December to February. The season of glass eels in the estuaries occurs late farther from the main Kuroshio Current. They occur in the mid-western estuaries of Korea mainly from March to May. It was reported that glass eels migrated into the Taiwan rivers at temperatures lower than 19 °C, and few appeared at temperatures over 19 °C[3]. Also, glass eels occur during the cold months in the southern Japanese estuaries. In the Keum River located at the mid western Korea, glass eels begin to appear in late February at temperature of <5 °C,

Lee, T. W and W. S. Kim (2001) Thermal effects on respiratory activities of glass eels at different developmental stages. J. Taiwan Fish. Res., **9**(1&2):129-136.

showing a peak in April. Few are collected in late May at temperatures over 16 °C.

The above results indicated that the glass eels occur in the estuaries of the regions during the cold months. Leptocephali migrated in the warm currents, and it is well known that the optimal temperature is around 25 °C during the growing stage in the continental water. This implies that the optimal temperature for eels may be different according to their growing stages.

Oxygen consumption rates of glass eels in different pigmentation stages were measured in different temperature and salinity regimes to determine the tolerant and optimal temperature ranges of glass eels.

Materials and Methods

Experimental animals

Glass eels were collected in the Keum River, mid-western coast of Korea in late March for the early pigmentation stage (VIA_I) and in late April for the late pigmentation stage (VIB). The pigmentation stages of the glass eels were determined after the criteria given by Bertin[4]. The collected glass eels were immediately transported to the KORDI laboratory and then kept in a holding tank of 50 liters without feeding and under constant laboratory conditions of 12-h light (L) and 12-h dark (D). The oxygen consumption rate (OCR) was measured at 2 experiments with 2 different staged glass eels. They were acclimated for 7 days before the experiment to the same condition in which they were collected; 8 °C and 32 psu for group - 1 (stage VIA_I) and 10 °C and 20 psu for Group - 2 (VIB)

Experimental design

In Group - 1 (VIA_I), the OCR measured at the salinity of 32 psu as the temperature is gradually raised from 8 to 26 °C (1 °C 24 h^{-1}) for 383.5-h (16 - d). In Group - 2 (VIB), the OCR was measured at the salinity of 10 and 20 psu as the temperature was gradually raised from 10 to 25 °C (1 °C 24 h^{-1}) for 255.2 - h (11 - d).

The OCR of the glass eels was measured by an automatic intermittent-flow-respirometer (AIFR - 1 system with 2 chambers) (Fig. 1). The measuring system was installed in a constantly darkened incubator (MLR - 350, Revco, USA). Seawater was filtered free of bacteria through a sterile membrane filter (with two Sartorius Capsule Filters, input 0.2 µm and output 0.07 µm) to reduce background oxygen consumption, due to bacteria, and the background oxygen consumption was measured by running blanks (i. e., no glass eels) for 12 - h before the experiments. Salinity (psu) was measured with a salinometer (LF 320, WTW, Germany). Experimental water was passed through the experimental chambers using a magnetic drive gear pump (Reglo - ZS, Ismatec SA, Switzerland). The animals are undisturbed, and subjected to a constant flow rate of water (345 $ml \cdot min^{-1}$) by a drive gear pump. Thick -walled Tygon tubings were used to connect the chambers to dissolved oxygen probes and a three-way actuator valve (TX 350 -1 DA - 2/1, Ilyoung, Korea) assembly which allowed the respirometer to operate in a open flow - through or closed mode. Oxygen levels in the plexiglas experimental chamber (300 m*l* for 3 glass eels) were maintained between 85% (lowest) and 95% (highest) saturation. When the oxygen level dropped below the predetermined limit, the drive gear pump and actuator valve supplied the system with saturated seawater until the selected oxygen levels were reached. The differences in oxygen solubility with decreasing salt content were calculated using a computer by Weiss[5]. The glass eels OCR was calculated from the changes in oxygen saturation levels in the test chambers with time. Saturation concentrations, KO_2 ($ml\ l^{-1}$), were calculated for standard conditions (atmospheric pressure P_{atm} = 1 atm = 1,013 mbar) as a function of temperature and salinity according to Weiss[5]. More detailed descriptions of calculation methods are given in Kim et al. [6,7].

Analysis of oxygen consumption records

Rhythmicity of the glass eel OCR was determined by a maximum entropy spectral analysis (MESA) program using raw data transformed into 30-min lag intervals. Time series were analyzed for periodicity using MESA spectra following the procedures and algorithms described by Dowse and Ringo[8]. Rhythmic analyses of the OCR were performed using the weighted smooth curve procedure of 2%. To plot a best-fit smooth curve through the center of the data, locally weighted least squares error methods were used (KaleidaGraphy custom program for Macintosh, Synergy Software). The value of 2% obtained from the repeated tests showed a best-fit curve. Statistical values were computed for each batch from the data points measured.

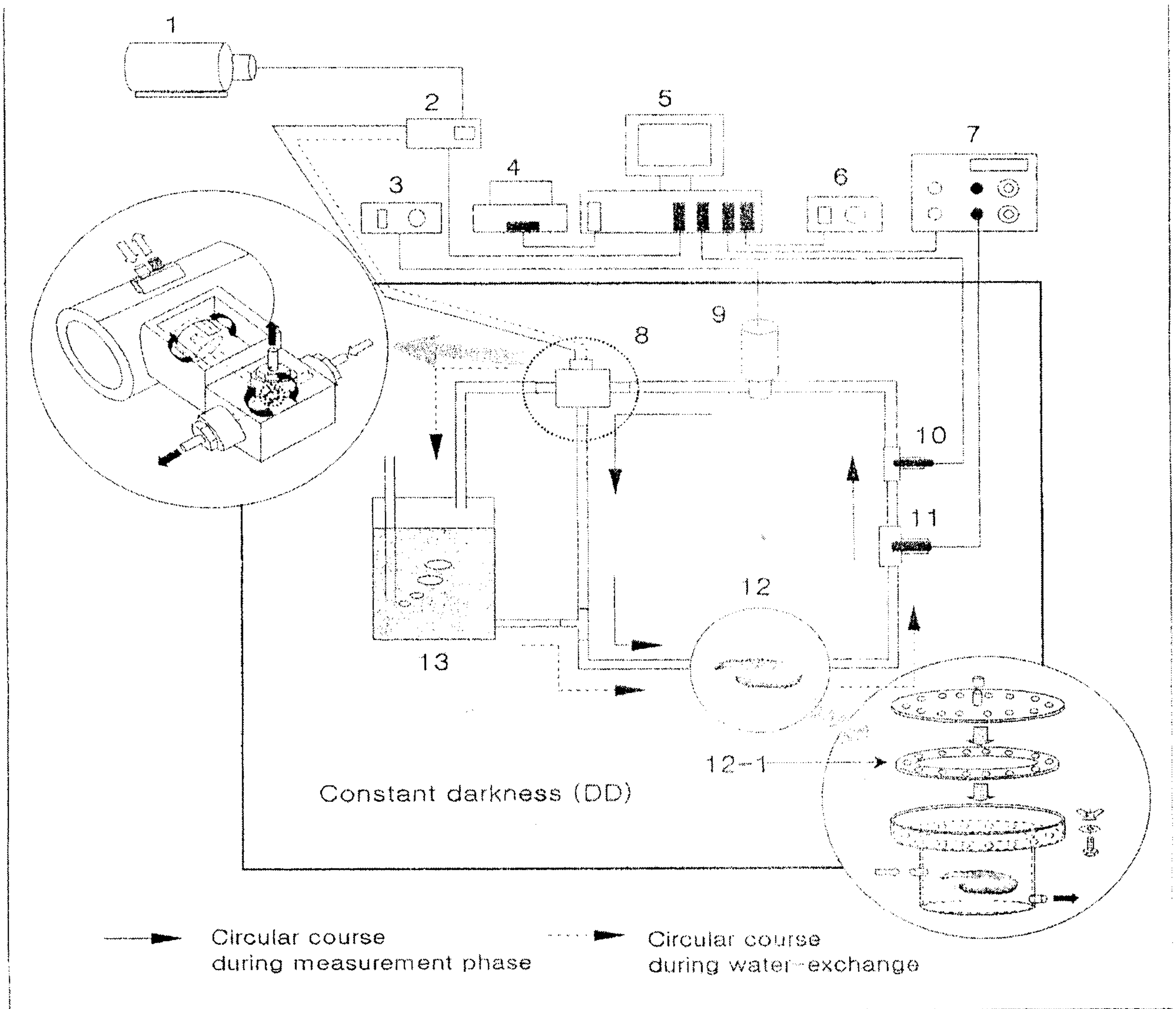

Fig. 1. Schematic diagram of the automatic-intermittent flow respirometer (AIFR) used to measure oxygen consumption of the glass eels *Anguilla japonica*. (1) compressor, (2) valve control box, (3) pump control, (4) printer, (5) computer for control and data storage, (6) air pressure sensor, (7) pico amperemeter, (8) three way valve, (9) toothed wheel pump, (10) temperature sensor, (11) oxygen sensor, (12) chamber, (12-1) silicon ring, (13) reservoir container.

Results

Group-1 (stage VIA_I)

The OCR for three glass eels measured for 383.5-h (16 - d) was fitted to a weighted smooth curve of 2% (Fig. 2). The mean OCR (mOCR) was highly variable, ranging from 0.07 to 0.28 m*l* $O^2 g^{-1}$ wt h^{-1}. The glass eels maintained relatively low oxygen uptake at mOCR of 0.10 ±0.06 (mean ±SD) m*l* O^2 g^{-1} wt h^{-1} until the temperature reached 12.9°C. The mOCR increased markedly from 12.9°C showing a peak (0.28 m*l* O^2 g^{-1} wt h^{-1}) at 21.5°C, and decreased thereafter. The replicated experiment for the glass eels in the stage VIA_I for 383.5-h showed a similar trend of OCR fluctuation as shown in Fig. 2 B.

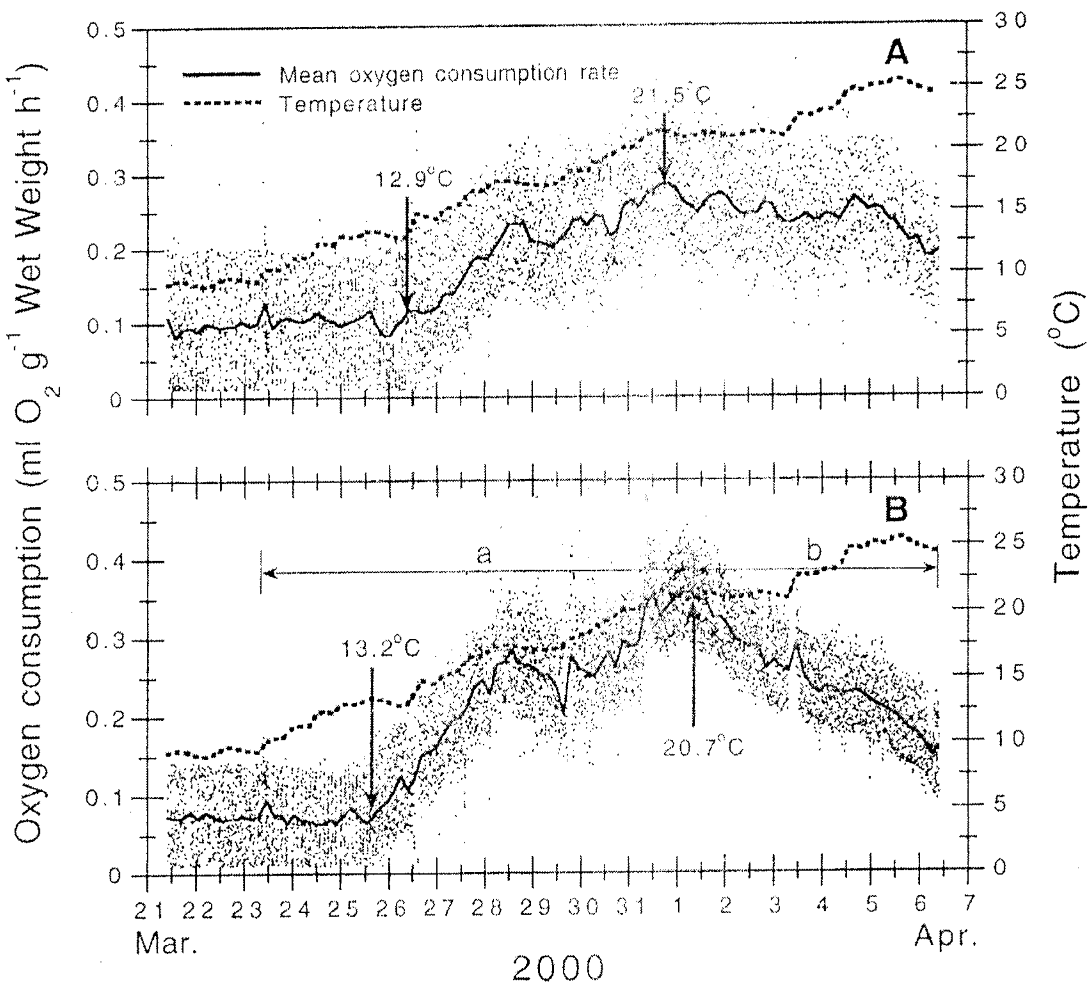

Fig. 2. (A) The time series of oxygen consumption by three glass eels (stage VIA_I) which were exposed to gradual temperature elevation from 8 to 26 ℃ at 31.2 psu for 383.5 - h. The arrows at 12.9 and 21.5 ℃ indicate the temperatures when the mOCR began to increase and showed a peak, respectively. Data points represent the mOCR during 90 - s intervals. (B) The replicated experiment.

MESA plots for the data presented in Figs. 2A and 2B indicated that the OCR peaks mainly occurred in 12.5 - h and 24.8 - h intervals, which corresponded to a semi-diurnal rhythm (Cqircatidal rhythm) and diurnal rhythm (Circalunidian or circadian rhythm) (Fig. 3). It indicated that on the 11 th day after the onset of the experiment where glass eels were subjected to <20.7 °C, the "clock oscillator" of glass eels changed from having a semi-diurnal (12.4 - h) to diurnal biological clock occurring at 24.8-h intervals.

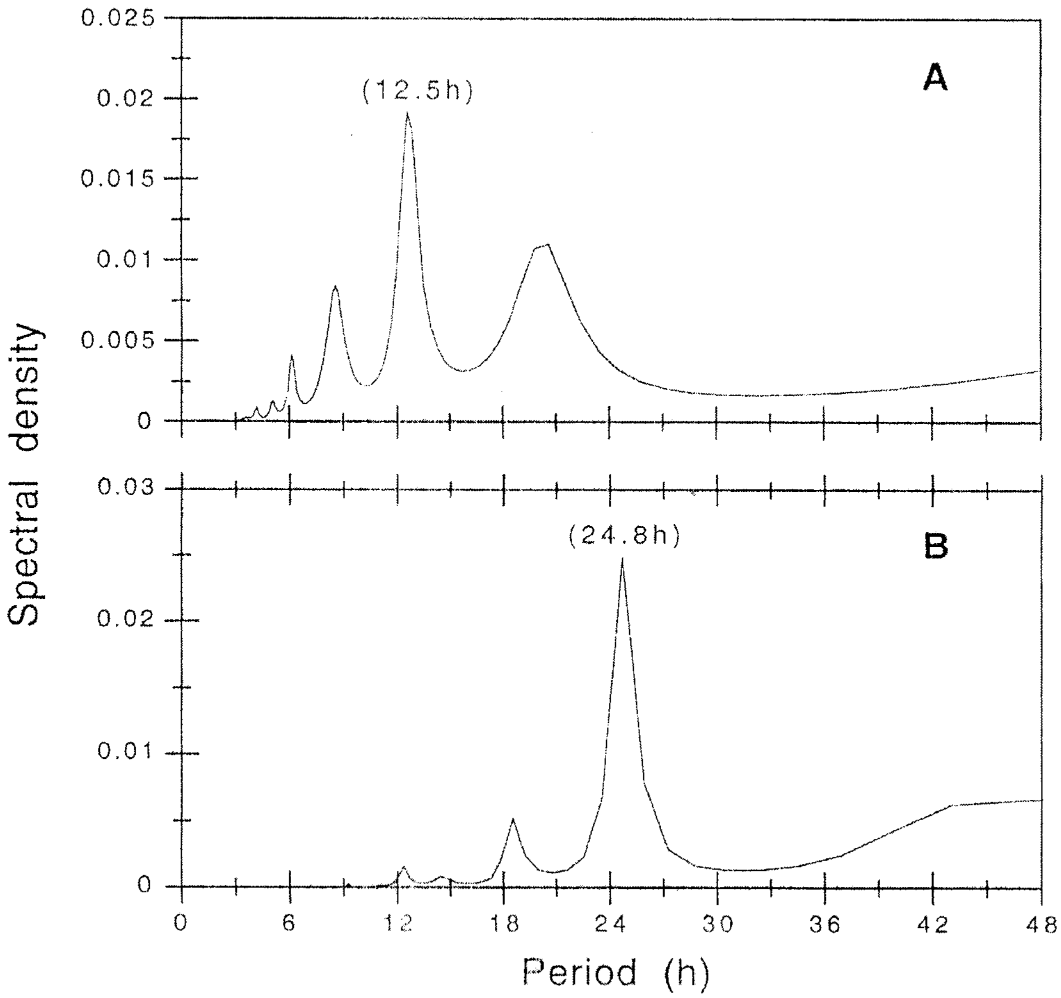

Fig. 3. Maximum Entropy Spectral Analysis (MESA) spectra for three glass eels at the temperature from 8 to 20.7 ℃ (the region of a in Fig. 2B) and over 20.7℃ (the region of b in Fig. 2B). Period length (h) corresponding to the dominant peaks in the MESA plots is given in parentheses.

Group - 2 (stage VIB)

The OCR of three glass eels was observed for 255.2 - h (10.6 - d) at 20 and 10 psu, and the results were fitted to a weighted smooth curve of 2% (Fig. 4). Magnitudes of mOCR were variable. The mOCR results of both data increased from 10°C. Magnitudes of OCR appeared to coincide with increasing temperatures at 1°C · 24 h - 1 in the experimental apparatus until the temperature reached 25°C. The mOCR numbers, averaged over the entire duration of the experiment and over the entire range of oxygen levels (between 85.4 and 94.7%), were 0.26 and 0.19 m*l* O^2 g^{-1} wt h^{-1} at 20 and 10 psu, under constant darkness, respectively. It was noticeable that the elvers in 20 psu consumed about 30.1% more oxygen than in 10 psu. No mortality was observed in 10 psu.

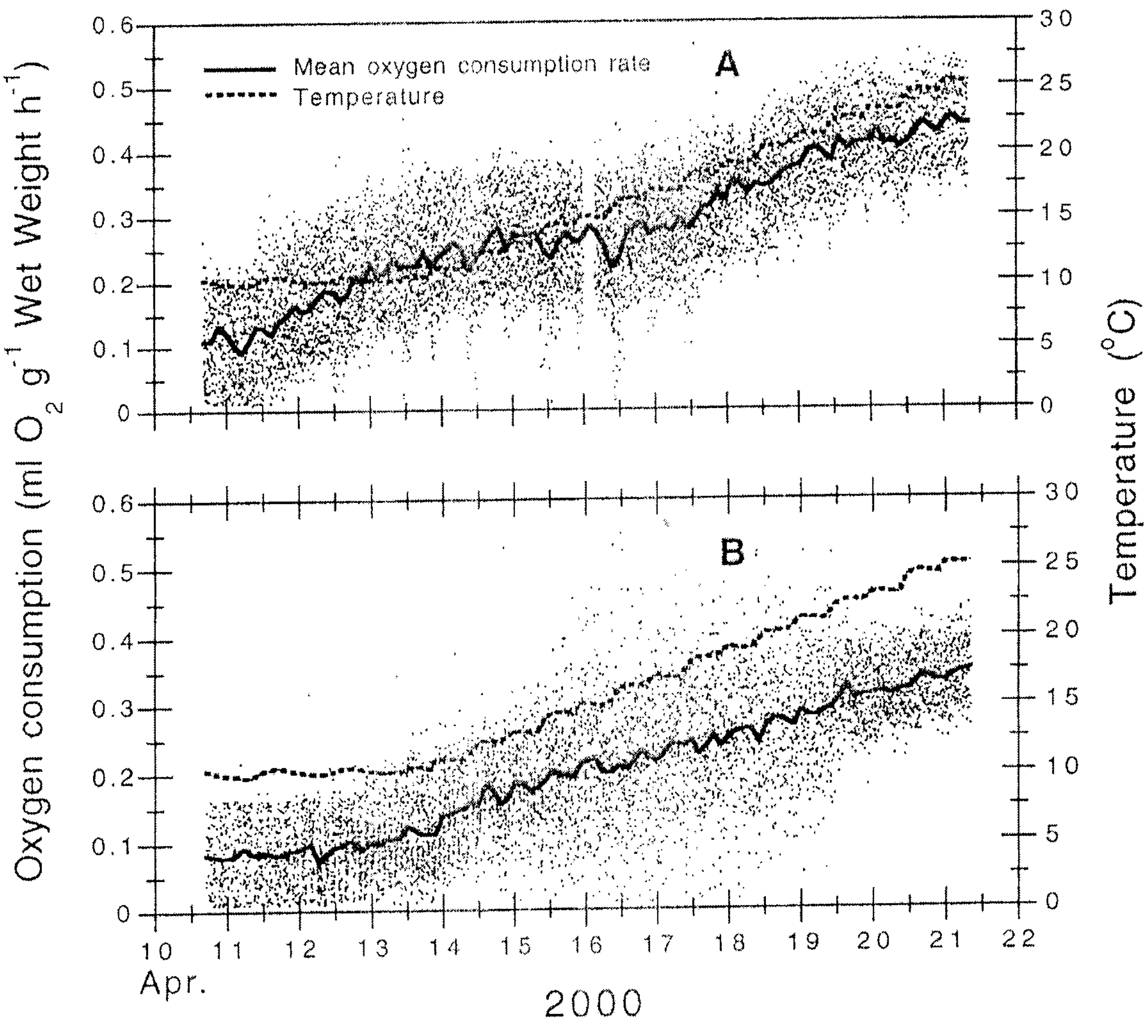

Fig. 4. The time series of oxygen consumption by three glass eels (stage VIB), which were exposed to gradual temperature elevation from 10 to 25 ℃ at 20.0 psu (A) and 10 psu (B) for 255.2 - h. Data points represent the mOCR during 90-s intervals.

MESA spectra of the data set presented in Fig. 4 indicated that the OCR peaks mainly occurred at 25.8- and 24.8 - h., which correspond with a circadian rhythm (Fig. 5). The instantaneous OCR also showed minor peaks in short periods of 8.9 and 11.8 - h intervals, respectively (Fig. 5).

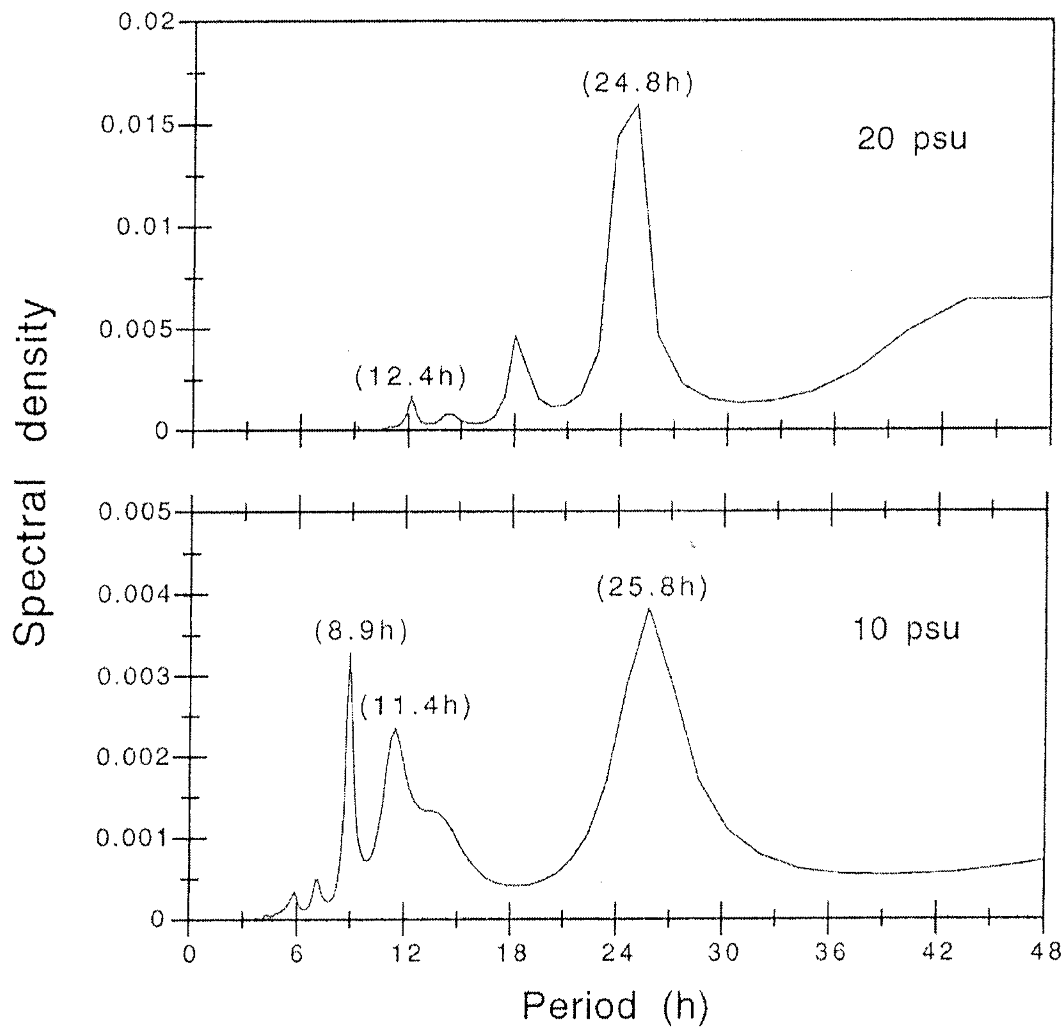

Fig. 5. Maximum Entropy Spectral Analysis (MESA) spectra for three glass eels at 20 psu (A) and 10 psu (B). Data presented in Fig. 4. Period length (h) corresponding to the dominant peaks in the MESA plots are given in parentheses.

Discussion

The leptocephali undergo some morphometric and functional changes during metamorphosis such as size diminution, advances of the anus and increase in head length[9]. Also, they do not to feed during the metamorphosis. These changes during metamorphoses of the anguillid eels were also expected but not yet confirmed in the laboratory. It is well known that the *Anguilla* glass eels collected at later stages were smaller than those collected earlier[10]. These data confirm at least partly that the glass eels also do not feed and survive with the preserved energy during migration over the continental shelf.

The OCR of the glass eels was constantly low at the temperature lower than 13°C in our experiments. (Fig. 2, 4). Glass eels occur in Korean estuaries at temperatures between 5 and 16 °C showing a peak around 10-13 °C. Glass eels collected early at the temperature lower than 10 °C were composed principally of early developmental stage (earlier than of VIA_I). These data indicate that the glass eels prefer to stay in cold waters (<13 °C) to save the energy without any external nourishment.

The OCR in the early pigmentation stage (stage VIA_I) gradually increased from 13 °C to 20 °C and decreased thereafter (Fig. 2). The rhythmic activity related to the local tidal events was clear in the OCR measurements by 20 °C, but indistinct at temperatures over 20 °C. The water temperature in the Taiwan estuaries was in the highest range for the *A. japonica* glass eels during the upstream migration. Glass eels did not appear at temperatures over 19°C in these estuaries[3]. These results confirmed that glass eels finished their upstream migration before the temperature reaches 19°C.

The OCR measurements continued to increase with temperature up to 25 °C for the eels in the late pigmentation stage (stage VIB). Energy consumption rate increased rapidly as temperature increased over 13 °C suggesting that the fish cannot live for a longer period of time only with internal energy reserve. The glass eels began to feed at temperatures over 15 °C[11]. We considered that glass eels developed rapidly to the growing stage at higher temperatures in order to feed.

The optimal temperature of *A. japonica* changed according to the growing stage. The leptocephali drift in the warm Kuroshio water, and the growing eels in the continental waters grow fast at temperature over 25°C. However, the glass eels are adapted to low temperature. The migration of glass eels during the cold months is considered to be one of the strategies to save energy when they do not feed.

Acknowledgments

We thank to S. W. Hwang and S. D. Hwang for their elver collection. This study was supported in part by Korea Maritime Institute (No. 20000137), and by the Ministry Science & Technology (MOST) of Korea under a project of the National Research Laboratory (NRL) to W. S. Kim.

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