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Determination of the Temperature-Dependent Index of Mitotic Interval (τ_o) for Chromosome Manipulation in Far Eastern Catfish *Silurus asotus*

In-Seok Park and Jae-Hyun Im*

Division of Ocean Science, College of Ocean Science and Technology,
Korea Maritime University, Pusan 606-791, Korea
* Faculty of Marine Life Science, Kunsan National University, Kunsan 573-701, Korea

Temperature-related cleavage rates and mitotic intervals (τ_o) in the far eastern catfish *Silurus asotus* were studied by averaging the duration of the second and third embryonic divisions to establish effective procedures for chromosome manipulations. At higher temperatures eggs developed faster and underwent more asynchronous development. Mitotic intervals for far eastern catfish were 21.5±1.4 min at 22°C, 18.5±1.2 min at 24°C, and 14.0±2.1 min at 26°C. There was a strong negative correlation between τ_o and water temperature ($Y = -1.85X + 21.9$, $R^2 = 0.9868$, where Y is τ_o and X is temperature).

Key words : Chromosome manipulation, mitotic intervals, *Silurus asotus*

The far eastern catfish *Silurus asotus* is a number of the typical freshwater Siluridae and an important commercial catfish in Korea (Kim *et al*., 1988; Kim, 1997). Although, various studies have been performed on specific aspects relating to far eastern catfish culture, few studies have been directed towards genetic improvements which will be determining factors in the future of far eastern catfish culture. Various chromosome manipulation methods are used to restore diploidy for gynogenesis and androgenesis to produce tetraploid population (Thorgaard, 1983). Efficacy of chromosome manipulation depends on accurately an applying an appropriate thermal shock timed to affect chromosome separation during from metaphase to anaphase (Saat, 1993).

Optimizing shock induction requires empirical determination of a shock's magnitude, duration, and time of application (Thorgaard, 1983). The optimal time of application depends on temperature, which affects the rate of embryonic development in poikilothermic species. A measure of developmental rate suggested by Dettlaff and Dettlaff (1961) is the duration of one mitotic cycle during early synchronous cell cleavage, or the interval between two consecutive cell divisions. This measure, τ_o or "Dettlaff unit" is expressed in minutes (Saat, 1993). The mitotic interval varies inversely with temperature and the relationship must be determined empirically; however, regressions of τ_o on temperature can be used as a basis for comparing species with similar spawning biology (Dettlaff, 1986).

In this study, therefore, we determined temperature-related cleavage rates or mitotic intervals (τ_o) to establish the efficient procedures for chromosome manipulation in this species. During the 1998 spawning season, mature broodfish were purchased, transported and maintained in Genetics and Breeding laboratory, Faculty of Marine Life Science, Kunsan National University, Korea. And three females and five males were selected and each sex was held separately in a 460 l bottom-filtered circular tanks supplied with temperature controlled freshwater. The flow rate was set at 10 l min^{-1} and the water temperature at 26±0.5°C. Milt was obtained by

hand-stripping, 24 hours after injection with human chorionic gonadotropin (HCG) at a dose of 1,000 IU per kg body weight. Milt from 5 ripe males was pooled to inseminate far eastern catfish eggs in vitro. Eggs were obtained by hand-stripping, 24 hours after injection with HCG at a dose of 2,000 IU per kg body weight. Considering the usual ranges for spawning and early development of far eastern catfish, cleavage frequency and mitotic intervals were determined at 3 different temperature of 22, 24 and 26°C. Freshly ovulated aliquots of 1,000 eggs from each female were pipetted into 3 replicate petri dishes and mixed with 2~3 ml milt which was diluted 40 times with physiological saline (128 mM NaCl, 26.8 mM KCl, 18 mM $CaCl_2$, 2.4 mM $NaHCO_3$, pH 7.0). Sperm activation was initiated by the addition of 26°C ambient freshwater. The fertilized eggs were incubated in 500 ml containers filled with ambient freshwater and supplied with aeration. Incubation temperatures were maintained using temperature controlled water baths set at 22, 24 and 26°C.

Samples of approximately 50 eggs were generally taken at 5 minute intervals over the period 30 minutes to 100 minutes post-activation from each replicate. However more frequent samples were taken as the anticipated time to first cleavage approached. Sampled embryos were examined at a 50X magnification to determine the developmental stage. Time to the first cleavage furrow was recorded, but was used only as the start for timing of the subsequent synchronous divisions. Time to the first division is not used in estimating τ_O because the interval from egg activation to first cleavage is two or more times the duration of subsequent synchronous divisions (Saat, 1993; Shelton and Rothbard, 1993). The time (minutes from activation) when 5 to 10% of the developing embryos reached the 4 (τ_{II}) and 8 (τ_{III}) cell stages was recorded. The value of 5 to 10% was selected based on the recommended of Dettlaff (1986). Mean mitotic cycle intervals (τ_o) were calculated as $\tau_o = \tau_{III} - \tau_{II}/2$. The relationships between mean mitotic interval and water temperature were examined by simple linear regression using SPSS.

Far eastern catfish eggs underwent cleavage over the temperature range of 22 to 26°C. As shown in Fig. 1, at the higher temperature the eggs of far eastern catfish showed the faster development. These results are similar to those of pearl oyster *Pinctada fucata martensii* reported by Komaru and Wada (1990). We observed the asynchronous development to first cleavage at all temperatures however this asynchronous development was more apparent at the lower temperatures. The synchrony of mitotic events is a critical factor to ensure efficient chromosome manipulation (Downing and Allen, 1987). Based on these results, we could predict that chromosome manipulations would be most efficieint at temperatures of 22 to 24°C.

In far eastern catfish as in case of some cyprinids, it is difficult to determine time of cleavage past the 8-cell stage (Shelton and Rothbard, 1993). For this reason we calculated τ_o using the average interval of the second and third cleavage furrows. Over the range of incubation temperatures we tested the relationship between tem-

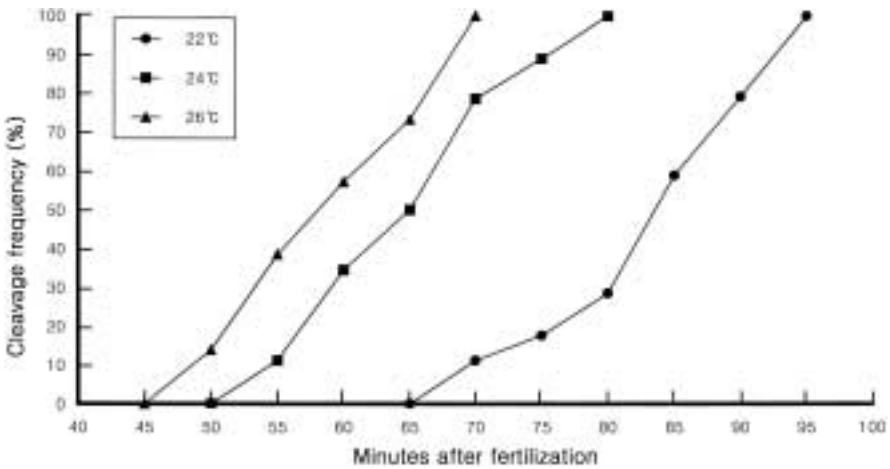

Fig. 1. The percentages of far eastern catfish eggs developed to anaphase of first cleavage at three different temperatures over time.

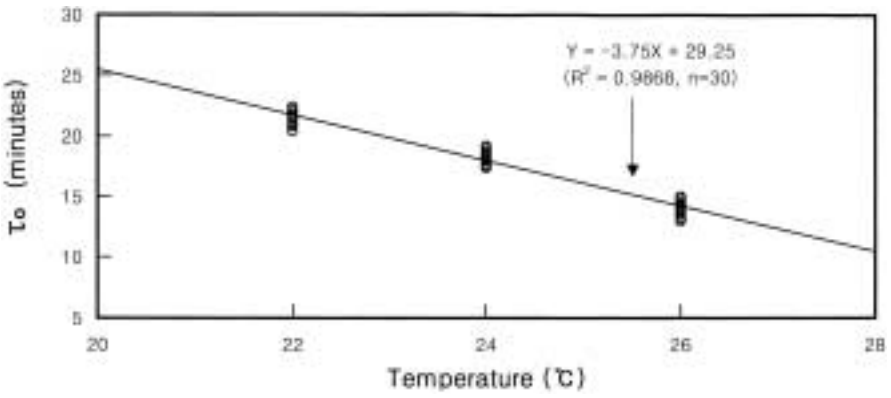

Fig. 2. Mitotic intervals (τ_o, Y) for far eastern catfish as a function of temperature (X). Eggs from 3 females were fertilized with pooled sperm from 5 males and were distributed among the temperature treatment.

perature and mitotic interval for far eastern catfish is best described by the linear relationship Y = $-1.85X+21.9$ ($R^2 = 0.9868$, n = 50)), where Y is τ_O and X is temperature in °C (Fig. 2). Mean mitotic intervals and standard deviations were 21.5 ± 1.4 min at 22°C, 18.5 ± 1.2 min at 24°C, and 14.0 ± 2.1 min at 26°C (Fig. 2). In fish the relationships between mitotic interval and water temperature are typically linear providing temperatures are within the range in which the species of fish naturally spawn and develop (Shelton and Rothbard, 1993). Far eastern catfish have much shorter mitotic intervals (τ_o) than other species at comparable temperatures (Shelton *et al*., 1997).

The results of this study conducted that obvious specific differences in time of egg development in far eastern catfish, demonstrated that temperature may be masked by variations in ambient temperature, which is one of the most potent influences on rate of development. While, optimization of treatment protocol for chromosome manipulation in most fishes proceeds through a long series of iterations, in which the effectiveness of several variables; type of shock; magnitude of shock; initiation and duration of shock is tested (Thorgaard, 1983). The Results obtained in this work will be helpful for chromosome manipulation by use of cleavage frequency data and τ_o data in far eastern catfish, and estimate the τ_o for efficient ploidy induction using temperature shock to at 22°C τ_o. However, following researches are required to test the environmental factors such as salinities and oxygen influence the rate of development in far eastern catfish (Blaxter, 1969).

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메기 *Silurus asotus* 염색체조작을 위한 온도 의존적 체세포분열 간격지수 결정

박 인 석 · 임 재 현

군산대학교 해양생명과학부

메기 *Silurus asotus*에서 염색체조작을 효과적으로 하기 위하여, 여러 온도 조건에서의 난할율과 제2, 제3 난할 시간의 평균화에 의한 체세포분열 간격지수(τ_o)를 조사하였다. 빠른 난 발생과 비동시적 발생은 고온에서 더욱 뚜렷하게 나타났다. 메기의 체세포분열 간격지수는 22°C에서 21.5±1.4분, 24°C에서 18.5±1.2분 그리고 26°C에서 14.0±2.1분이었다. 3가지 수온조건과 τ_o 간에는 $Y = -1.85X + 21.9$ ($R^2 = 0.9868$, Y는 τ_o 그리고 X는 수온)의 직선회귀가 성립되었다.