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A Temperature-Dependent Index of Mitotic Interval (τ_0) in *Haliotis gigantea* and *Haliotis discus*

In-Seok Park[1*], Jae Hyun Im[2], Young-Don Lee[3], Bong-Lae Kim[4] and Seock-Jung Han[5]

[1]Division of Ocean Science, Korea Maritime University, Busan 606-791, Korea
[2]Development of Fishery Sciences, Kunsan National University, Kunsan 573-701, Korea
[3]Marine and Environment Research Institute, Cheju National University, Jeju 695-810, Korea
[4]Taean Marine Hatchery, National Fisheries Research and Development Institute, Taean 357-945, Korea
[5]Bukjeju Marine Hatchery, National Fisheries Research and Development Institute, Jeju 695-835, Korea

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ABSTRACT

In order to establish effective procedures for chromosome manipulation in *Haliotis gigantea* and *H. discus*, which are of enormous aquacultural potential, temperature-dependent measures of mitotic intervals (τ_0) were determined. Mitotic intervals (τ_0) in these abalone were determined by averaging the duration of the first and third embryonic divisions over a range of temperatures from 8 to 26°C. The relationships of each mitotic interval at two cell (τ_I), four cell (τ_{II}), eight cell (τ_{III}), sixteen cell (τ_{IV}) and τ_0, to temperature (T in °C) in *H. gigantea* were log τ_I = 176.1 - 28.3T, log τ_{II} = 199.5 - 12.4T, log τ_{III} = 236.2 - 12.2T, log τ_{IV} = 269.3 - 14.1T and log τ_0 = 83.1 - 32.8, respectively. The relationships of each mitotic interval at τ_I, τ_{II}, τ_{III}, τ_{IV} and τ_0, to temperature in *H. discus* were log τ_I = 104.9 - 13.8T, log τ_{II} = 138.3 - 10.5T, τ_{III} = 172.4 - 10.2T, log τ_{IV} = 211.3 - 12.2T and log τ_0 = 85.6 - 33.3T, respectively. There were strong, negative correlations between mitotic interval and water temperatures for all ten temperatures in these two species (*H. gigantea*: Y = -138.75 logX + 341.25, R^2 = 0.97; *H. discus*: Y = -112.33 logX + 255.22, R^2 = 0.98, where Y is mitotic interval and X is temperature).

Key words : Temperature-dependent, mitotic interval, *Haliotis gigantea*, *Haliotis discus*.

INTRODUCTION

Abalones are low-moving inhabitants of rocky intertidal areas and are easy prey for fishermen. Thus their numbers have been decimated in all the countries mentioned (Viana, 2002). Recently, emphasis was shifted to hatchery propagation in the South, still with the intention of stocking to augment the fishery. Experimental work was initiated at the Bokjeju Marine

* To whom correspondence should be addressed.
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Hatchery, National Fisheries Research and Development Institute at Jeju island, Korea in 1977 and has since been taken up at several other laboratories (Bang, 1977). At least six species of abalone are found along Korean shores: *Haliotis discus hannai, H. discus, H. diversicolor aquatilis, H. diversicolor diversicolor, H. gigantea*, and *H. madaka*. Of these six, the greatest success has been had in rearing *H. discus hannai*, a Northern species and *H. discus*, a Southern species (Pyen, 1970; Bang, 1977).

Although, various studies have been performed on specific aspects related to abalone aquaculture, few studies have been directed towards genetic improvements which will be determining factors in the future of abalone aquaculture. Various chromosome manipulation methods are used to restore diploidy for gynogenesis to produce tetraploid population (Thorgaard, 1983; Arai et al., 1984; Okumura et al., 1998). The ability to effectively manipulate ploidy through the application of suitable shocks (temperature, pressure or chemical) early in egg development requires empirical determination of shock's magnitude, duration, and time of application (Thorgaard, 1983; Saat, 1993; Im et al., 2001).

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_0 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_0 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_0) to establish the efficient procedures for chromosome manipulation in these species.

MATERIALS AND METHODS

Selected specimens of the *Haliotis gigantea* and *H. discus*, sampled from the Cheju Island, and reared at the Bukjeju Marine Hatchery, National Fisheries Research and Development Institute, Jeju, Korea, were used to obtain egg and sperm specimens. Spawning for each species was induced by placing animals in sterilized and heated sea water. Eggs collected form one or two each species of females, pooled in a plastic tank containing about two liters of sea water, were inseminated by mixing with sea water suspension of sperm taken from one or two males. Relative concentration between eggs and spermatozoa was adjusted by applying different volumes of sperm suspension. Ambient water temperature for eggs, sperms, and zygotes was maintained approximately at 20 ± 1°C during spawning and insemination.

After being inseminated, eggs were exposed to different water temperatures, ranging from 8 to 26°C to examine the association between water temperature and time lags in developmental process of the beginning of the first cleavage. Samples of approximately 100 eggs from each species of replicate were generally taken and preserved in Ringer's solution containing 5% acetic acid at 2 min intervals over the period of 15 min to 12 hrs post-activation. However, more frequent samples were taken as the anticipated time to first cleavage approached.

Sampled embryos from each species were examined at a 50X magnification to determine stage of development. The time of appearance of the first cleavage furrow was recorded, and this was used as the start for timing of the subsequent cell divisions. The time (minutes from activation) when approximately 10% of the developing embryos reached the 2 (τ_I) and 8 (τ_{III}) cell stages was recorded. The value of 10% was selected based on the recommended of Ignatyeva (1975). Mean mitotic cycle intervals (τ_0) were calculated as $\tau_0 = (\tau_{III}-\tau_I)/2$. The relationship between mean mitotic interval and water temperature was examined by simple linear regression using SPSS.

RESULTS AND DISCUSSION

Eggs of *Haliotis gigantea* and *H. discus* underwent cleavage over the temperature range of 8 to 26°C. At the higher temperature the eggs of these abalones developed faster. These results are similar to those reported by Komaru and Wada (1990), who reported faster egg development at the higher temperature of pearl oyster *Pinctada fucata martensii*. We observed the developmental variation between initiation of first cleavage stage between eggs at all temperatures. However, this developmental variation was more apparent at the lower temperatures (Table 1, Fig. 1 and 2). The identity of mitotic events is a critical factor to ensure efficient chromosome manipulation (Downing and Allen, 1987).

The duration of one mitotic cycle during the period of identical cell divisions (τ_0) has proved to be an appropriate unit to compare the duration of development processes at

Table 1. The relationship of τ_I, τ_{II}, τ_{III}, τ_{IV} and τ_0 to temperature as described by $\log(\tau_n) = a + yT$ (*T*. temperature in °C) in *Haliotis gigantea* and *H. discus*.

Species	τ_n	Constant *a* (±S.E.)	Regression coefficient *y* (±S.E.)	R^2	Level of significance	Sample size
Haliotis gigantea	τ_I	176.1±23.1	-28.3±13.9	-2.0399	<0.0001	100
	τ_{II}	199.5±30.7	-12.4±18.4	-0.6696	<0.0001	100
	τ_{III}	236.2±36.4	-12.2±21.9	-0.5568	<0.0001	100
	τ_{IV}	269.3±42.1	-14.1±25.3	-0.5557	<0.0001	100
	τ_0	83.1±8.9	-32.8±5.4	-6.1232	<0.0001	100
H. discus	τ_I	104.9±17.4	-13.8±10.5	-1.3157	<0.0001	100
	τ_{II}	138.3±23.7	-10.5±14.2	-0.7369	<0.0001	100
	τ_{III}	172.4±29.6	-10.2±17.8	-0.5757	<0.0001	100
	τ_{IV}	211.3±34.9	-12.2±21.0	-0.5796	<0.0001	100
	τ_0	85.6±4.7	-33.3±2.8	-11.7833	<0.0001	100

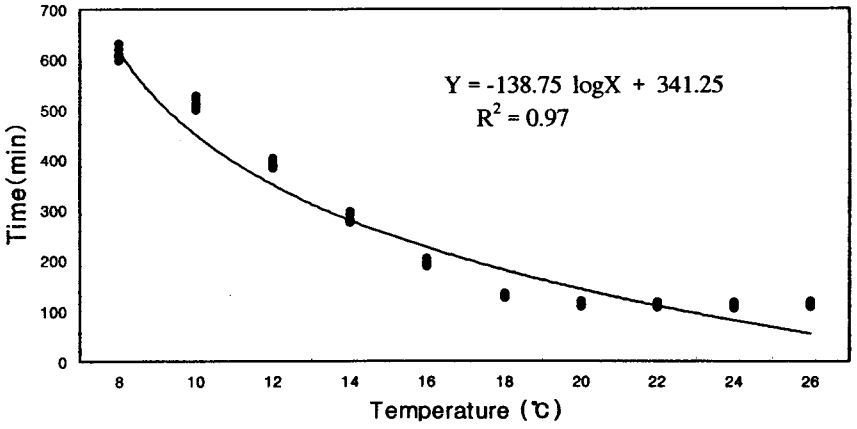

Figure 1. The empirical values of $\tau_0 = (\tau_{III}-\tau_I)/2$ for *Haliotis gigantea* within the temperature of 8 to 26°C. The curves have been drawn by inspection.

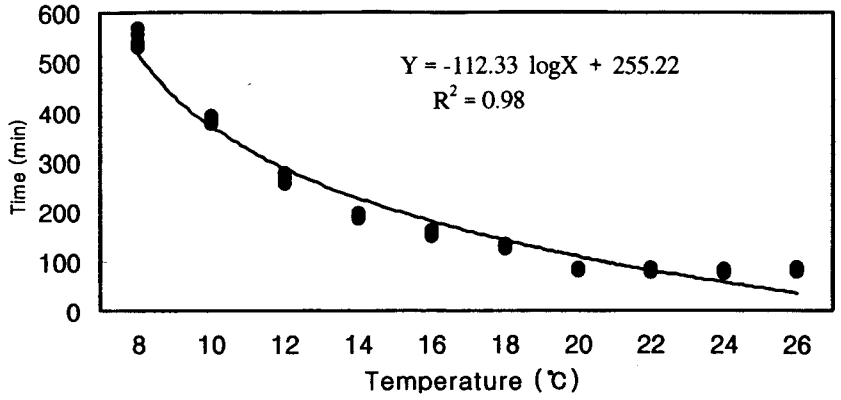

Figure 2. The empirical values of $\tau_0 = (\tau_{III}-\tau_I)/2$ for *Haliotis discus* within the temperature of 8 to 26°C. The curve has been drawn by inspection.

different temperatures in poikilothermic animals undergoing identical cell divisions during their early development (Dettlaff, 1986; Saat and Veersalu, 1996). In *H. gigantea* and *H. discus* as is the case in 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_0 using the average interval of the second and third cleavage furrows.

Table 1 shows the relationships of each mitotic interval at two cell (τ_I), four cell (τ_{II}), eight cell (τ_{III}), sixteen cell (τ_{IV}) and τ_0, to temperatures in *H. gigantea* and *H. discus*, respectively Over the range of incubation temperatures, the relationship between temperature and mitotic interval for *H. gigantea* and *H. discus* were best described by the linear relationship Y = -138.75 logX + 341.25 (R^2 = 0.97, n = 100)), Y = -112.33 logX + 255.32 (R^2 = 0.98, n = 100), respectively, where Y is τ_0 and X is temperature in °C (Fig. 1 and Fig. 2). The temperature dependence of τ_0 in abalones used in this experiment is similar to those in far eastern catfish *Silurus asotus* (Park and Im, 2001), winter flounder *Pseudopleuronectes americanus* (Park and Johnson, 2002) and perch *Perca fluviatilis* L. and ruffe *Gymnocephalus cernuus* L. (Saat and Veersalu, 1996).

In fish the relationships between mitotic interval and water temperature are typically curvilinear providing temperatures are within the range in which the species of fish naturally spawn and develop (Saat and Veersalu, 1996). Available data suggest that the curves of τ_0 dependence on temperature are highly species-specific (Shelton et al., 1997). This species-specificity of the rate development may be useful in distinguishing the taxonomic range of different grouping of abalone. The distribution of data in *H. gigantea* and *H. discus* appears linear over the range of 8 to 26°C (Fig. 1 and Fig. 2). This linear response of the plot for τ_0 against temperature is accordance with the study on the developmental rate for black carp over the range of 20 to 30°C (Shelton and Rothbard, 1993). However, additional observations are obviously needed. Abalones are known to have a broad range of thermal tolerance with respect to spawning and egg incubation (Fujino et al., 1984).

The results of this study conducted that obvious specific differences in time of egg development abalones, 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 mollusca proceeds through a long series of literations, in which the effectiveness of several variables; type of shock; magnitude of shock; initiation and duration of shock is tested (Thorgaard, 1983, Kim et al., 2001).

The Results obtained in this work will be helpful for chromosome manipulation by use of cleavage frequency data and τ_0 data in *H. gigantea* and *H. discus*, and estimate the τ_0 for efficient ploidy induction using temperature shock to at temperatures between 20 and 26°C τ_0. However, following researches are required to test the environmental factors such as salinities and oxygen which influence the rate of development in *H. gigantea* and *H. discus* (Blaxter, 1969).

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