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Determination of the temperature-dependent index of mitotic interval (τ_O) for chromosome manipulation in winter flounder *Pseudopleuronectes americanus*

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Abstract

In order to establish effective procedures for chromosome manipulations in the winter flounder *Pseudopleuronectes americanus*, temperature-dependent measures of mitotic intervals (τ_0) were determined. Mitotic intervals (τ_0) were determined by averaging the duration of the first and third embryonic divisions over a range of temperatures from 0 to 20 °C. At higher temperatures, eggs developed faster and underwent more identical development. Mitotic intervals for winter flounder were 98.5 ± 1.3 min at 0 °C, 79.5 ± 1.7 min at 5 °C, 59.5 ± 1.7 min at 10 °C, 38.7 ± 1.8 min at 15 °C and 23.9 ± 2.2 min at 20 °C. There were strong, negative correlations between mitotic interval and water temperature for all five temperatures application ($Y = -19.00X + 117.40$, $R^2 = 0.9969$, where Y is mitotic interval and X is temperature).

Keywords: Temperature-dependent; Mitotic interval; *Pseudopleuronectes americanus*

1. Introduction

The winter flounder, *Pseudopleuronectes americanus*, is a member of the order Pleuronectiformes and the family Pleuronectidae, which contain many commercially important flatfish species. This species is distributed in inshore waters along the Atlantic Coast of North America from Georgia to Newfoundland and Labrador (Liem and Scott, 1966; Witherell and Burnett, 1993). In addition to being an important commercial and recreational

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fish species, this species is also considered to be a good potential candidate for use in commercial scale aquaculture (Litvak, 1999). Winter flounder are a very hardy disease resistant fish species capable of living over a wide range of temperatures and salinities. These features which make this species attractive for use in aquaculture also makes this species a good 'model fish' for use in scientific studies (Douglas et al., 1999; Litvak, 1999). The relatively small genome of winter flounder, the availability of an EST data base, and the fact that molecular probes developed for this species cross-hybridize readily with other flatfish and to a lesser extent, other fish species makes this species valuable for use in molecular and genomic studies (Douglas and Gallant, 1998; Douglas et al., 1999).

The production of polyploid fish (e.g. triploids) and fish with uniparental inheritance (gynogenetes) has important applications in both aquaculture and fish research (Mair, 1993). The ability to effectively manipulate ploidy through the application of suitable shocks (temperature, pressure or chemical) early in egg development requires empirical determination of a shock's magnitude, duration, and time of application (Thorgaard, 1983). In poikilothermic species such as fish, the time of application is of course dependent on temperature and the ploidy manipulation.

In this study, we determined temperature-related cleavage rates or mitotic intervals, measured as the "Dettlaff unit" (τ_0) for winter flounder in order to establish the efficient procedures for chromosome manipulation. The Dettlaff unit is the duration in minutes of one mitotic cycle during early synchronous embryonic cleavage, or the interval between two consecutive cell divisions (Saat and Veersalu, 1996b; Shelton et al., 1997). When measured over a range of temperatures, the relationship of τ_0 to temperature as determined by regression analysis can be used to predict developmental events that are influenced by temperature within a species and between species with a similar spawning biology (Dettlaff, 1986). To date, mitotic intervals (τ_0) have been used to estimate optimal times for chromosome manipulation in a variety of species such as the paddlefish (*Polyodon spathula*), shovelnose sturgeon (*Scaphirhynchus platorynchus*), common carp (*Cyprinus carpio*), tench (*Tinca tinca*), and the black crappie (*Pomoxis nigromaculatus*) (Flajšhans et al., 1993; Shelton and Rothbard, 1993; Mims et al., 1997; Shelton et al., 1997; Gomelsky et al., 2000). In our laboratories, we have found the use of τ_0 to be very useful in optimizing time for chromosome manipulations in winter flounder and other species (Im et al., 2001).

2. Materials and methods

Winter flounder gametes were obtained from wild caught broodstock maintained at the Aquaculture Research Station, Institute for Marine Biosciences, National Research Council, Halifax, Canada. During the 1999 spawning season, three females and five males were selected and each sex was held separately in 2000-l circular tanks supplied with temperature-controlled seawater. The flow rate was 30 l min^{-1} and the water temperature was 5 °C. Milt was obtained from five ripe males by hand-stripping, 24 h after injection with human chorionic gonadotropin (HCG, Sigma, USA) at a dose of 1000 IU per kg body weight. The milt was pooled and used to inseminate winter flounder eggs in vitro.

Eggs were obtained by hand-stripping, 24 h after injection with HCG at a dose of 2000 IU per kg body weight. For the determination of cleavage frequency and mitotic intervals

at five different temperatures, freshly ovulated aliquots of 2200 to 2500 eggs from each female were pipetted into three 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 5 °C ambient seawater. The fertilized eggs were incubated in 500-ml containers filled with ambient seawater (28–30 ppt salinity) and supplied with aeration. Incubation temperatures were maintained using temperature controlled water baths set at 0, 5, 10, 15 and 20 °C.

Samples of approximately 50 eggs from each replicate were generally taken and preserved in Ringer's solution containing 5% acetic acid at 15 min intervals over the period of 15 min to 11 h post-activation. However, more frequent samples were taken as the anticipated time to first cleavage approached. Sampled embryos were examined at a 50 × magnification to determine the 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 recommendation 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.

3. Results and discussion

Winter flounder eggs underwent cleavage over the temperature range of 0–20 °C. At the higher temperature, the eggs of winter flounder developed faster. These results are similar to those reported by Saat and Veersalu (1996a,b), who reported that faster egg development at the higher temperature of some teleost species. We observed the developmental variation between the initiation of first cleavage stage between eggs at all temperatures, however, this developmental variation was more apparent at the lower temperatures (Fig. 1). 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 different temperatures in poikilothermic animals undergoing identical cell divisions during their early development (Dettlaff, 1986; Saat and Veersalu, 1996b). In winter flounder 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. Over the range of incubation temperatures, the relationship between temperature and mitotic interval for winter flounder is best described by the linear relationship $Y=-19.00X+117.40$ ($R^2=0.9969$, $n=50$), where Y is τ_0 and X is temperature in °C (Fig. 2). The temperature dependence of τ_0 in winter flounder is similar to those in Baltic herring *Clupea harengus membras* L. (Saat and Veersalu, 1996a) and perch *Perca fluviatilis* L. and ruffe *Gymnocephalus cernuus* L. (Saat and Veersalu, 1996b).

Mean mitotic intervals and standard deviations were 98.5 ± 1.3 min at 0 °C, 79.5 ± 1.7 min at 5 °C, 59.5 ± 1.7 min at 10 °C, 38.7 ± 1.8 min at 15 °C and 23.9 ± 2.2 min at 20 °C (Fig. 2). In fish, the relationships between mitotic interval and water temperature are

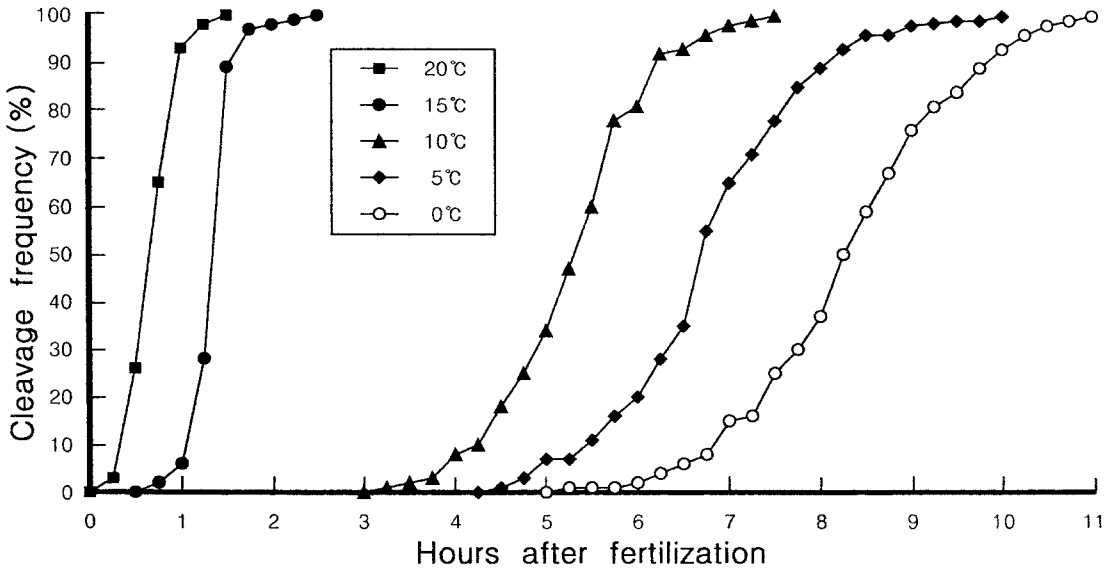

Fig. 1. The percentages of winter flounder eggs developed to anaphase of first cleavage at five different temperatures overtime.

typically curvilinear providing temperatures are within the range in which the species of fish naturally spawn and develop (Saat and Veersalu, 1996b). The distribution of data appears linear over the range of 0–20 °C (Fig. 2). This linear response of the plot for τ_0 against temperature is in accordance with the study on the developmental rate for black carp over the range of 20–30 °C (Shelton and Rothbard, 1993). However, additional observations are obviously needed. Winter flounder are known to have a broad range of thermal tolerance with respect to spawning and egg incubation (Lee and Litvak, 1996).

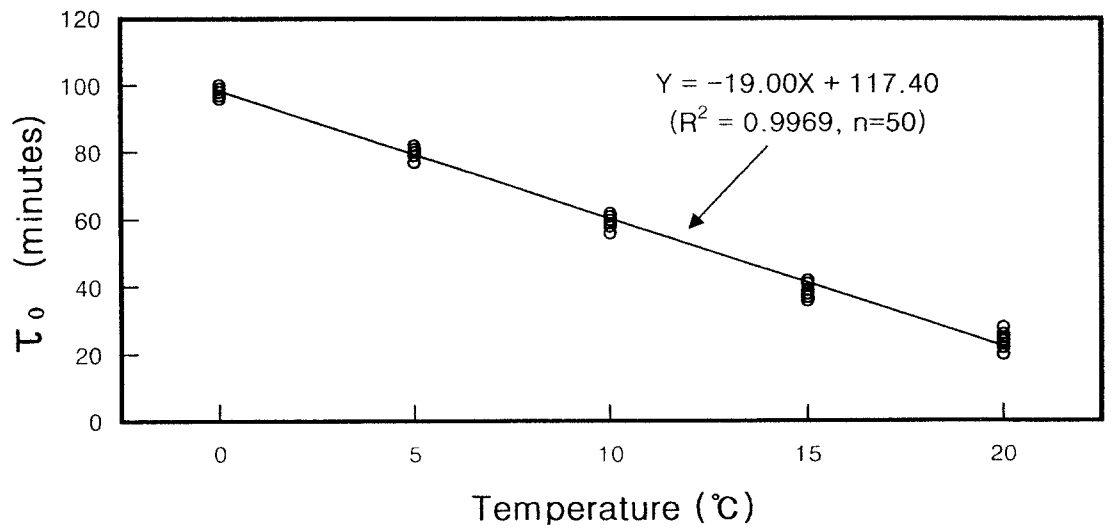

Fig. 2. Mitotic intervals (τ_0, Y) for winter flounder as functions of temperature (X). The temperatures used are within the normal range for spawning and early development in this species. Eggs from three females were fertilized with pooled sperm from five males and were distributed among the temperature treatments.

The relative rate of development in winter flounder exceeded that of Baltic herring normally reproducing at temperatures between 0 and 5 °C, and was remarkably lower than in perch and ruffe between 10 and 20 °C (Saat and Veersalu, 1996a,b). 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 fish. Considering the identity of mitotic events and short time intervals (τ_0), we would predict chromosome manipulations in winter flounder would be most efficient at temperatures between 15 and 20 °C.

This study demonstrated an obvious specific differences in time of egg development at different temperature in winter flounder. The optimization of treatment protocols for chromosome manipulations in fish is usually time consuming as a number of variable such as type of shock, magnitude of shock, timing and duration of shock need to be determined (Shelton et al., 1997). The cleavage frequency data and τ_0 data obtained during our study will be useful for the development of an optimal of treatment protocol for chromosome manipulation in this winter flounder.

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