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A Temperature-Dependent Index of Mitotic Interval (τ_0) for Chromosome Manipulation in Paddlefish and Shovelnose Sturgeon

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Abstract.—A temperature-dependent measure of the mitotic interval (τ_0) can help standardize chromosome manipulation in fish eggs. A tau unit (τ_0) is the duration in minutes of one mitotic cycle during synchronous embryonic cleavage. It is measured over a range of temperatures, and the resulting relationship of τ_0 to temperature can be used to anticipate developmental events that are affected by temperature. Optimum induction of chromosome manipulation requires development of a specific treatment of egg shocking for each species. Timing of shock is a critical variable, but pretreatment incubation temperature affects the rate of development and thus the optimum absolute time for shocking. Mitotic intervals (τ_0) are reliable indicators of developmental rates over normal temperatures for egg incubation, and thus can be used to estimate optimal times for chromosome manipulation. Mitotic intervals for paddlefish *Polyodon spathula* and shovelnose sturgeon *Scaphirhynchus platorynchus* were estimated by averaging the duration of the second and third embryonic divisions (two-cell to four-cell and four-cell to eight-cell stages). Mitotic intervals (τ_0) for paddlefish ranged from 74 ± 2.8 min (mean ± SD) at 16°C to 52 ± 1.4 min at 20°C; τ_0 for shovelnose sturgeon was 66 ± 2.5 min and 45 ± 1.1 min at these temperatures.

Chromosome manipulation is an important technique in aquaculture and fish management. Various methods are used to restore diploidy for gynogenesis and androgenesis or to produce triploid and tetraploid populations. The principal techniques involve shocking eggs early in development with sharp temperature changes (up or down), increases in hydrostatic pressure, or chemical treatments (Purdom 1983; Nagy 1987). Shocking eggs to retain the second polar body (polar-body or early shock) is used for gynogenesis or triploidization, whereas shock to interrupt the first mitotic division (endomitotic or late shock) can be used for gynogenesis, tetraploidization, or restoration of diploidy in the induction of androgenesis (Thorgaard 1983). Efficacy of ploidy manipulation depends on accurately applying an appropriate shock timed to affect chromosome separation during metaphase–anaphase (Gomelskiy et al. 1989; Saat 1993).

Optimizing shock induction requires empirical determination of a shock's magnitude, duration, and time of application (Thorgaard 1983; Shelton 1987, 1989). 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). When time to a particular developmental stage (τ_n, minutes) at a particular temperature is divided by τ_0 for that temperature (τ_n/τ_0), the dimensionless quotient should be valid for all normal incubation temperatures, because developmental rates depend on mitotic rates, which also facilitates comparisons among species (Dettlaff and Dettlaff 1961; Dettlaff 1991; Saat 1991). Developmental reference points can be any identifiable stanza or stage in ontogeny. The quotient (as a coefficient) can be multiplied by an empirically determined τ_0 to obtain the time (minutes) to a particular developmental stage (at which shocking should be applied, for example) at a particular incubation temperature. Use of the coefficient thus incorporates an adjustment for temperature-affected rate differences (Gomelskiy et al. 1989; Cherfas et al. 1990, 1993; Flajshans et al. 1993; Rothbard and Shelton 1993; Shelton and Rothbard 1993; Rothbard et al. 1997).

Paddlefish *Polyodon spathula* and shovelnose sturgeon *Scaphirhynchus platorynchus* are chondrostean fishes in the order Acipenseriformes. They are commercially important fishes valued for their meat but mainly for their roe, which is produced as caviar (Vasetskiy 1971; Carlson and Bonislawsky 1981; Mims et al. 1993; Sternin and Dore 1993). World caviar supply is under threat because of anthropogenic environmental degradation, and within some of the sturgeons' natural range, political strife has interrupted regulation of fishing and repopulation of sturgeon stocks through artificial propagation. Therefore, future caviar production may increasingly depend upon aquaculture, in which case production of all-female stocks will be economically beneficial. Chromosome manipulation and steroid-induced sex reversal can be integrated to develop culture systems that produce monosex female stocks (Nagy and Csanyi 1984; Bye and Lincoln 1986; Shelton 1986). Development of such culture systems is the impetus for our ongoing studies with paddlefish and shovelnose sturgeon (Mims et al. 1995).

The objective of this study was to measure temperature-related cleavage rates, or mitotic intervals (τ_0), of paddlefish and shovelnose sturgeon. The empirically derived tau curves are being used in our studies to develop chromosome manipulation protocols for these two species. The interval between egg activation and the application of shock in chromosome manipulation of acipenseriforms is considerably longer than those for most teleosts (Dettlaff 1986, 1991; Saat 1993; Shelton and Rothbard 1993). Therefore, differences in preshock incubation temperature affect the absolute time of meiotic and mitotic activity to a relatively greater extent for acipenserids. Cleavage of acipenserid embryos is holoblastic, but although early embryonic development has been described for paddlefish (Ballard and Needham 1964; Bemis and Grande 1992), no such information is available for shovelnose sturgeon. Further, although early embryogenic rates have been reported for some sturgeons (Ginsberg and Dettlaff 1991), no information on temperature-related early mitotic rate has been published for either of the species described here.

Methods

During the spawning seasons of 1994–1996, mature paddlefish and shovelnose sturgeon were caught in the Ohio River, transported to the Aquaculture Research Center of Kentucky State University, and held in 2.5-m-diameter circular tanks. Female and male broodstocks of paddlefish and sturgeon were held in separate tanks, which were supplied with dechlorinated water at a flow rate of 12 L/min. Water temperature was 18 ± 0.3°C. The males of both species were given single injections of luteinizing hormone releasing hormone analog (LHRH-A: des-Gly10[D-Ala6]-LHRH) at a rate of 0.05 mg/kg. Females were given a total dose of LHRH-A at the rate of 0.1 mg/kg; sturgeon received a single injection, but paddlefish were given two injections (0.1 and 0.9 of the total dose) separated by 12 h (Graham et al. 1986). Paddlefish ovulated 12–14 h after the last injection and sturgeon ovulated 18–24 h postinjection; males of both species were actively spermiating within 12–18 h.

Ovulated eggs from each female were stripped into a dry pan and milt from one conspecific male was added and mixed. An aqueous suspension of Fullers earth was mixed with the eggs to activate the spermatozoa and to prevent egg adhesion; the clay suspensions were immersed in separate water baths at each temperature to be tested. The experimental temperature range was 14–21°C for paddlefish and 16–25°C for shovelnose sturgeon. These temperature ranges correspond to the published optima for spawning and early development of each species (Wallus et al. 1990). The pans were floated in water at the test incubation temperature while the eggs were stirred for about 10 min. Several hundred eggs were then loaded into each of several screened incubation units and maintained at the various discrete temperatures (±0.3°C) in aerated water baths. The relationship between temperature and mitotic interval was examined only within the temperature range optimal for reproduction. Paddlefish do not begin to spawn until the water temperature has exceeded 12–14°C (Russell 1986) and shovelnose sturgeon begin spawning at 16–18°C (Christenson 1975). At short intervals,

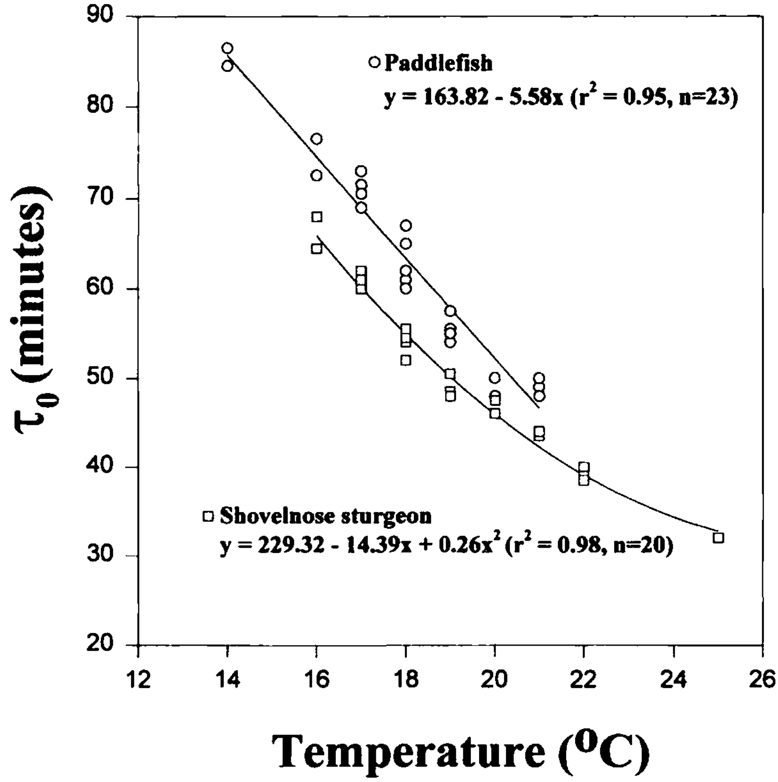

FIGURE 1.—Mitotic intervals (τ_0, y) for paddlefish and shovelnose sturgeon as functions of temperature (x) within the usual ranges for spawning and early development. Each datum represents a cross of one female × one male; eggs from some crosses were distributed among several temperature treatments.

20–30 eggs were removed from each temperature treatment and examined live at a magnification of 45× for progression of development; eggs were examined more frequently as anticipated time to first cleavage approached. 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_0 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). Mean mitotic cycle intervals (τ_0) were calculated from the average of the two subsequent cleavages (two to four and four to eight cells) based on the first 5–10% of eggs that cleaved, as recommended for acipenseriform fishes by Dettlaff (1991). The relationships between mean mitotic interval and water temperature were examined by general linear model (GLM) procedures (SAS Institute 1985). Eggs from eight paddlefish and seven shovelnose sturgeon females were used in this study; 23 estimates of mitotic interval (τ_0) were made at seven temperatures for paddlefish and 20 estimates were made at eight temperatures for shovelnose sturgeon.

Results

The mitotic interval decreased with increasing temperature for both species (Figure 1). The data for paddlefish were best described by a linear relationship; shovelnose sturgeon data were slightly curvilinear. Mitotic intervals for paddlefish were significantly longer ($P \leq 0.05$) than those for shovelnose sturgeon within the range of temperatures examined. Mean (calculated) mitotic intervals (τ_0) and standard deviations for paddlefish were 74 ± 2.8 min at 16°C, 63 ± 2.9 min at 18°C, and 52 ± 1.4 min at 20°C (Table 1; Figure 1). Shovelnose sturgeon τ_0 intervals at the same temperatures were 66 ± 2.5, 55 ± 1.5, and 45 ± 1.1 min, respectively.

Discussion

Relationships between mitotic interval (τ_0) and water temperature are typically linear within the

TABLE 1.—Comparison of mitotic intervals (τ_0) in minutes for representative acipenseriform and cypriniform fishes.

Species[a]	τ_0 at temperatures (°C) of: 16	18	20	22	Source
Paddlefish	74	63	52	41	Present study (calculation)
Shovelnose sturgeon	66	55	45	39	Present study (calculation)
Russian sturgeon	56	49	45	39	Ginsberg and Dettlaff (1991)
Common carp	50	36	30	23	Shelton and Rothbard (1993)
Tench	48	37	30	23	Flajshans et al. (1995)

[a] Russian sturgeon *Acipenser gueldenstaedti*; common carp *Cyprinus carpio*; tench *Tinca tinca*.

usual spawning and developmental temperatures but curvilinear if temperatures outside these ranges are used (Dettlaff and Vassetzky 1991; Shelton and Rothbard 1993). The paddlefish data appear to be within the range of more normal development; shovelnose sturgeon data embraced a wider range of temperatures, some of them probably somewhat beyond the optimal for development. For acipenseriforms, τ_0 can be estimated by the interval between the appearance of the first and second (2-cell to 4-cell) cleavage furrows for the first 5–10% of the eggs (Dettlaff 1991); for teleosteans, τ_0 is more often taken as one-half of the interval between the appearance of the second and fourth (4-cell to 16-cell) cleavage furrows (Ignatyeva 1975; Penaz et al. 1983). However, Shelton and Rothbard (1993) used the average interval of the second and third cycles (2 cells to 8 cells) for several cyprinids because of the difficulty in judging the time of cleavage in living material beyond the 8-cell stage. Saat (1993) emphasized the importance of basing the interval on successive karyokineses instead of on cytokinetic transitions. The metaphase–anaphase stages of nuclear division have the greatest importance for chromosome manipulation. However, estimation of the mitotic interval (τ_0) based on the appearance of consecutive cleavage furrows should provide interval estimates similar to those derived from consecutive nuclear divisions. The developmental duration is longer for acipenserids than for most teleostean fishes at comparable temperatures (Saat 1993). In Table 1, τ_0 for three acipenseriform and two cyprinid species are compared at four temperatures. The mitotic interval for paddlefish, shovelnose sturgeon, and Russian sturgeon ranged from 56 to 74 min at 16°C, compared to 48–50 min for common carp and tench. In contrast, within the lower optimal temperature range for spawning and development of rainbow trout *Oncorhynchus mykiss*, τ_0 is 300 min at 6°C and 220 min at 8°C (Ignatieva 1991).

Chromosome manipulation studies for gynogenesis or triploidization have been published for common carp and tench (Shelton and Rothbard 1993; Flajshans et al. 1995) and studies are in progress for the two North American acipenseriform species. At an incubation temperature of 18–20°C, the optimal time of early shock application (τ_s) for these two cypriniform species is approximately 3 min postactivation or about 0.1 τ_0 (3 min/30 min = 0.1 τ_0). Early shock time for paddlefish eggs incubated at 18°C is about 18 min postactivation, or 0.28 τ_0 (18 min/63 min = 0.28 τ_0) and shock time at 20°C should be initiated at about 15 min after activation (0.28 × 52 min = 14.6 min; authors' unpublished data). We have just completed preliminary gynogenesis trials for the shovelnose sturgeon. Based on the tau curve reported here and the similarity of reproduction to paddlefish, we assumed that the shock tau (τ_s) and other induction parameters for paddlefish would approximate those for shovelnose sturgeon. We were successful in producing high numbers of diploid gynogenote sturgeon in our first effort and now we will be able to refine the treatment in the upcoming season.

Optimization of treatment protocol for chromosome manipulation of 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. We have found that this process can be greatly facilitated through the use of τ_0 data and information on related species. Vasetskii (1967) attempted to manipulate ploidy for the Russian sturgeon, but shocked at only 3–6 min postactivation. Such early shocking is in the range of polar-body shock time for most teleosteans, but it is far too early for acipenseriforms. Based on the τ_0 data for the Russian sturgeon (Ginsberg and Dettlaff 1991), we think that optimal shock time for this species would be about 10–13 min postactivation at 18°C. Further, referencing shock time to mitotic interval may permit more meaningful comparison between species. For example, optimum shock time to induce triploidy in rainbow trout eggs is 15–25 min postactivation at 6–8°C (Diaz et al. 1993), which is similar to absolute time of shock for acipenserids, but in terms of Dettlaff units, shock application is at 0.05–0.11 τ_0. This range of τ_s is comparable to that for other teleosts.

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