Environmental Biology of Fishes Vol. 14, No. 1, pp. 43–50, 1985

Effect of temperature on early development of white and lake sturgeon, *Acipenser transmontanus* and *A. fulvescens*

Yuan L. Wang[1], Frederick P. Binkowski[2] & Sergei I. Doroshov
[1] *Department of Animal Science, University of California, Davis, CA 95616, U.S.A.*
[2] *Center for Great Lakes Studies, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, U.S.A.*

Keywords: Chondrostean, Acipenserid, Embryos, Larvae, Rate of development, Survival, Temperature adaptations

Synopsis

The effect of constant incubation temperatures (between 10° C and 26° C) on the developmental rates was found to fit a similar exponential relationship in both the lake and white sturgeon embryos and larvae. Although the lake sturgeon had an overall slower rate of development than the white sturgeon, no statistically significant difference was detected in the slopes of the exponential equations describing the effect of temperature on developmental rate. The effect of these incubation temperatures on embryonic survival also did not differ between these two species. Both species exhibited optimal survival between 14–17° C and incipient mortalities occurred at 20° C. Temperatures above 20° C were lethal for white sturgeon embryos. No effect of low incubation temperature on survival was evident from this study. A comparison of these North American species with Eurasian acipenserids suggests that all the sturgeon that have been examined exhibit a similar influence of incubation temperature on developmental rate.

Introduction

We examine the effect of temperature on the timing of early development and survival of two North American Acipenserid species: white sturgeon, *Acipenser transmontanus*, from San Francisco Bay, and lake sturgeon, *Acipenser fulvescens*, from the Great Lakes. These two species were abundant and commercially important in the past century but their numbers and harvest have dramatically declined due to overharvesting, pollution and dam construction (Harkness & Dymond 1961, Miller 1972). Recent investigations have shown that temperature is an important factor in the success of the artificial propagation of sturgeon (Binkowski & Czeskleba 1980, Doroshov et al. 1983). Understanding the environmental requirements, specifically temperature and how it effects development, growth and survival during the early life history stages, is essential for the successful hatchery management of sturgeon.

Detlaf et al. (1981) investigated the effect of temperature on cleavage and organogenesis in three acipenserid species from the Caspian and Azov seas. No similar information is available for North American sturgeon species. Normal stages of early development were described at ambient temperature (14 to 20° C) for the Atlantic sturgeon (Dean 1895), lake sturgeon (Harkness & Dymond 1961), white sturgeon (Beer 1980) and paddlefish (Ballard & Needham 1964).

* Contribution No. 277 Center for Great Lakes Studies, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA.

Material and methods

The observations were conducted in spring 1983 at the University of Wisconsin-Milwaukee (lake sturgeon) and at the University of California-Davis (white sturgeon). Zygotes of white sturgeon were obtained from a single mating. Broodfish were caught during the spawning run in the Sacramento River, then induced to ovulate by the administration of common carp pituitary extracts. Eggs of lake sturgeon were collected from two naturally ovulated females captured on spawning grounds below Eureka Dam on the Fox River, Wisconsin. Gamete collection, insemination and egg deadhesion procedures were similar for both species (Doroshov et al. 1983), and carried out in fresh water of 13–15° C.

The fertilized eggs and embryos which emerged were incubated at different constant (±0.5° C) temperatures until yolk absorption. Post fertilization time and survival at the developmental stages described below, were determined for both species.

Experimental procedures differed for lake and white sturgeon, and data obtained should be treated accordingly. Six temperature treatments: 11° C, 14° C, 17° C, 20° C, 23° C, 26° C, with four replications, were applied for white sturgeon. Approximately 1000 eggs in each replicate were incubated in conical plastic jars with an upwelling water flow. Jars were installed inside 15 liter round tanks, supplied with fresh ground water in six independent temperature controlled blocks. Emerging embryos escaped via outflows from the jars into the tanks and remained there until the end of the experiment. Eggs of lake sturgeon were incubated 'en masse' in commercial MacDonald jars at constant temperatures 10° C, 15° C, and 20° C, without replication. Embryos that emerged were held in insulated fiberglass tanks supplied with water of the same temperature. In both cases, water was aerated and dissolved oxygen was maintained at saturation.

We attempted to sample the embryos and larvae of both species at certain developmental stages, classified by Detlaf & Ginzburg (1954), as follows: stage 6 – third cleavage at animal pole; 14 – horizontal blastopore furrow; 22 – closure of neural tube; 29 – differentiation of S-shaped heart; 35 – initiation of hatching; 36 – completion of hatching; 40 – separation of intestine and stomach; 44 – completion of yolksac absorption, discharge of pigment plug from spiral valve. These stages are described in the 'Results' section.

Development of white sturgeon was monitored by 'in vivo' microscopic examination of 15–20 animals at 1 to 12 hour intervals, depending on stage and treatment. When the proportion of animals reaching the above stages was ≥50% of total sample, the post fertilization time was recorded and 50 to 80 specimens were sampled from each replicate and preserved in buffered formalin.

'In vivo' monitoring of lake sturgeon was not done. Instead, lake sturgeon were sampled at frequent intervals (every 4 hours for embryos before and every 20 hours after hatching, preserved in buffered formalin and later examined. The samples which contained >50% of the sturgeon at stages 6 and 40 were missing from one or more temperature treatments and these stages were deleted from the final data analysis.

Preserved specimens were examined and photographed under a dissecting microscope and the percentage of normally developing animals was counted in each sample. This was assumed to express the survival prior to hatching (all embryos that emerged survived from hatching to the end of the experiments). Data were pooled for the stages 6–14 (cleavage to gastrulation) and stages 22 through 36 (neurulation to hatching), since no difference in proportions of normally developing embryos was observed within these two groups.

The relationships between the timing of development and temperature were computed for each stage using exponential equations. The relationship between survival and temperature was fitted by least squares regression. All regressions yielded significant F values (≤0.05) and R^2 values ranged from 0.935 to 0.999.

Results

Embryonic development

Developmental stages, used for sampling during these experiments, are illustrated by photographs (preserved specimen) in Figure 1 and 2. Zygotes of white sturgeon are 4 mm in diameter and darkly pigmented with melanine. Eggs of lake sturgeon are smaller (3.5 mm) and lighter in color (brownish grey). Eggs of both species exhibit identical patterns of holoblastic cleavage. At the stage of third cleavage (CLVG, #6), the first cleavage furrow completely divides the egg. The second cleavage furrow traverses slightly beyond the equator of the egg, and the two third cleavage furrows are restricted to the animal hemisphere. The latter consists of eight micromeres, slightly unequal in shape and size. Bright white coloration retains the configuration of the faint grey crescent, present in both species prior to and during early cleavage stages.

At gastrulation the embryonic development of both species proceeds in similar fashion. The dorsal lip of the blastopore can first be observed as a faint pigmented line appearing slightly above the equator. Involution progresses as the lip elongates into

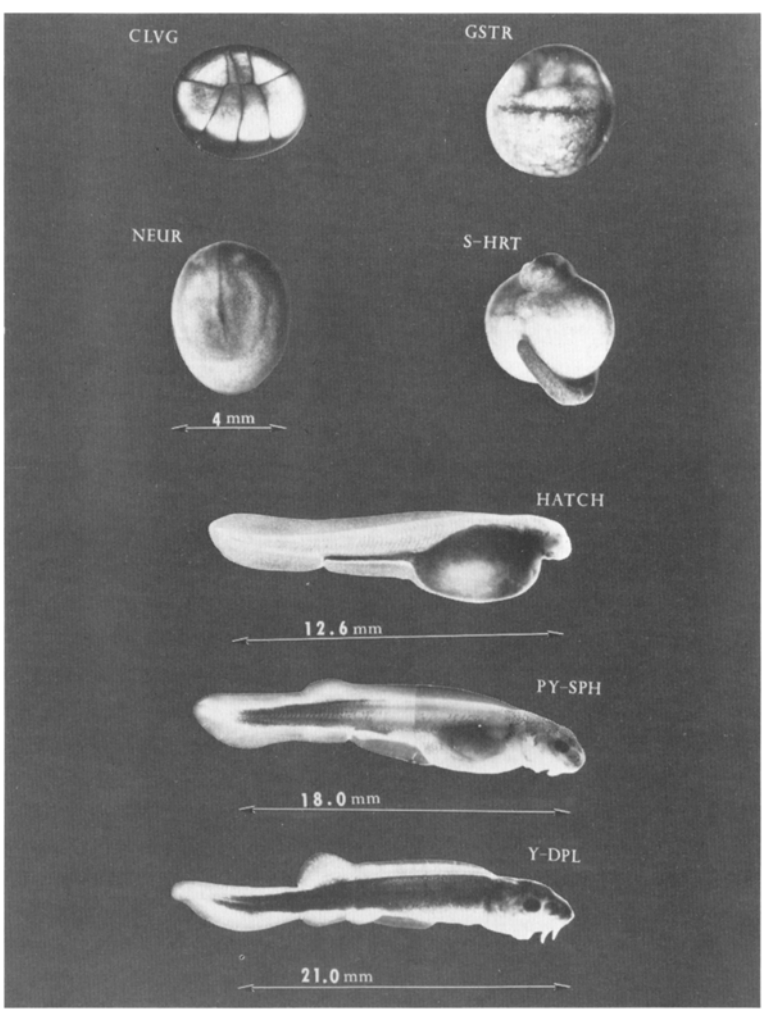

Fig. 1. Stages of embryonic development of white sturgeon, *Acipenser transmontanus:* CLVG – early cleavage, 8 micromeres (6); GSTR – early gastrula, horizontal blastopore (14); NEUR – late neurula, closure of neural tube (22); S-HRT – differentiation of S-shaped heart (29); HATCH – emerged embryo (35 and 36); PY-SPH – separation of intestine and stomach, differentiation of pyloric sphincter (40); Y-DPL – completion of yolk absorption (44).

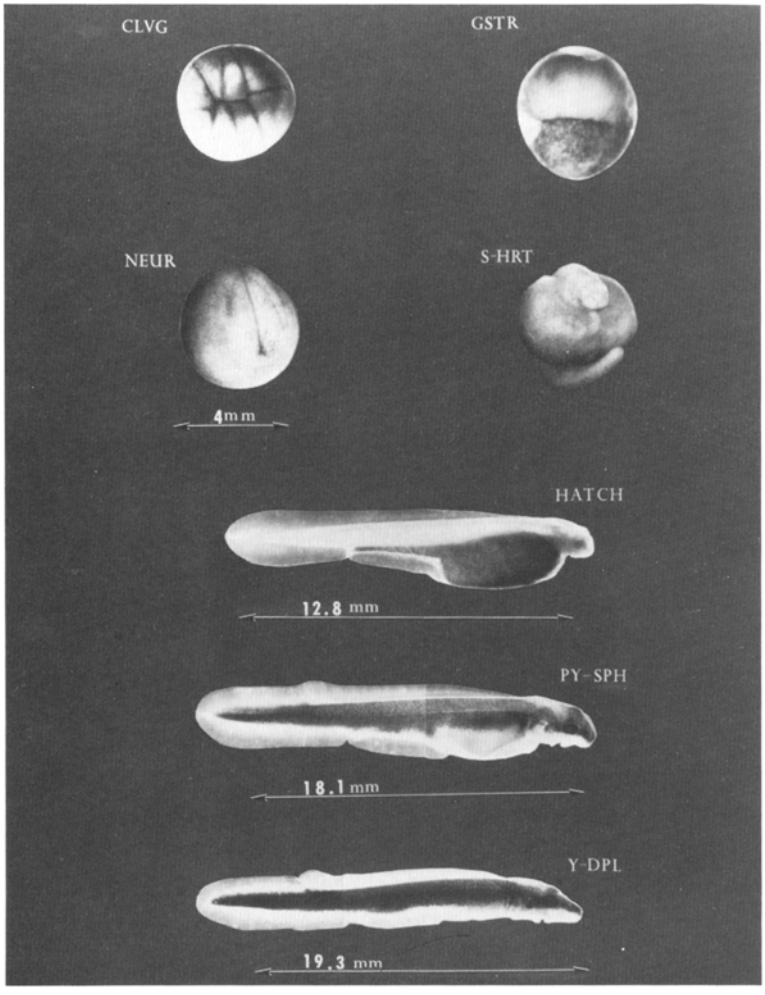

Fig. 2. Stages of embryonic development of lake sturgeon, *Acipenser fulvescens*. See Figure 1 for abbreviations.

the horizontal furrow (GSTR, #4) and the ectodermal cell mass moves toward the vegetal pole. Light or almost white colored blastoderm later surrounds dark endodermal cells resulting in the 'blastopore plug' stage, similar in both species. Before blastopore closure, the faint neural plate with pigmented folds extends from near the blastopore to almost half way around the egg. The neural folds move towards and join each other to form the neural tube. Neural fold closure first occurs at the mid section of embryo and then extends toward the cephalic region and toward the tail (NEUR, #22). The entire region is elevated and the first somites can be seen during the final period of neurulation.

The primordial heart appears as a straight tube projection below the head region of the embryo at the completion of neural tube closure. Following completion of neurulation, the heart tube flexes, assuming an 'S' shaped form. At this stage, the head region is elevated and the tail region is separated from the yolksac (S-HRT, #29).

As organogenesis continues, the tail elongates beyond the head region and the embryo begins to move. A pink colored substance (apparently associated with a release of hatching enzyme) appears in the perivitelline space a few hours prior to hatching. Hatching continues from one to several days, depending on water temperature. Embryos of white and lake sturgeon, collected at the beginning

and completion of hatching (HATCH, #35 and 36) were similar in their appearance and size (10 to 11 mm, total length). Newly emerged embryos of lake sturgeon have light brown coloration and a more elongated yolksac. White sturgeon embryos have dark pigmentation and an almost round, dark colored yolksac.

The latter in sturgeon is composed of yolk endoderm which forms the gastrointestinal tract. The posterior (intestinal) portion of yolksac is depleted first. As the intestinal region of yolksac reduces in size, its color changes from greyish (in white sturgeon) or brown (in lake sturgeon) to white and a narrow connection, the primordial pyloric sphincter, clearly divides the yolksac into two regions: the intestine, void of yolk material (with an exception of oil droplets), and the future gastric region, still filled with dark yolk (PY-SPH, #40). Differences in body shape, finfold differentiation and pigmentation are now apparent between the two species. Lake sturgeon have a more streamlined configuration, an undifferentiated finfold and a faint longitudinal band of melanophores extending from snout to caudal region. White sturgeon appear more robust, their dorsal, caudal and pelvic fins are differentiating, a darkly pigmented band extends through only the caudal region, from slightly behind the anus to almost the end of the notochord.

As development advances, the yolk material eventually becomes depleted and replaced with the differentiated gastric region. The gut region is retracted and streamlined with the rest of the body. The pigmented 'melanine plug' appears in the spiral intestine and is soon discharged through the anus prior to the initiation of exogenous feeding (Y-DPL, #44). At this point white and lake sturgeon are clearly distinct in their appearance and morphological features; in white sturgeon all fins are differentiated, including the heterocercal caudal fin, and the dorsal row of scutes is differentiating. Metamorphic processes in lake sturgeon are delayed. Instead, larvae of this species assume the appearance of a burrowing form; the finfold is not differentiated, the body is elongated and the snout points downwards. Darkly pigmented bands extend along both sides of the body, from the tip of snout to the end of the notochord.

Timing of the development

Post-fertilization times required to reach the above described stages at the experimental temperatures are given in Table 1. Approximately two-fold acceleration in the development is evident as early as at gastrulation (stage #14) in both species, at temperatures 10° to 20° C. Acceleration further increases in more advanced stages, especially in lake sturgeon. Lake sturgeon development appears to be slower, compared with that of white sturgeon, particularly in the lower temperature range. Embryos of white sturgeon emerge at 230–311 hours post-fertilization at 11° C and at 84–98 hours at 20° C. Hatching of the lake sturgeon was observed at 380–430 hours at 10° C and 90–105 hours at 20° C. White sturgeon reached the yolk depletion stage at 708 hours post-fertilization at 11° C, while lake sturgeon reached a similar stage at 1316 hours post-fertilization at 10° C.

Exponential equations describing the relationship between developmental time and temperature are given in Table 2. Slopes of the exponential lines (term 'b') tend to become more negative in more advanced embryonic stages, except for the white sturgeon late stages (Table 2). We found no statistically significant difference in terms 'b' among all stages of white and lake sturgeons, except for the last stage #44 'b' term was significantly more negative in lake sturgeon, $P<0.05$). However, all 'a' coefficients, characterizing elevation of curves, were significantly ($P<0.05$ to <0.001) greater in lake sturgeon, reflecting a slower rate of development in this species.

Survival curves were similar for both species (Fig. 3). All embryonic mortalities occurred between the early gastrula and late neurula. Range of temperatures between 12° C and 16° C appear to be optimal for embryonic survival in both species. Higher temperatures ranging from 20–22° C for embryos during cleavage and from 18–20° C for embryos during organogenesis showed lowered survival. The low temperature tolerance limits for both species are, apparently, below 10° C and were not evident in this study.

Table 1. Times required to reach various developmental stages and observed survival of white and lake sturgeon at the experimental temperature ranges.

White sturgeon							Lake sturgeon		
Temperature °C	11	14	17	20	23	26	10	15	20
				Time (hours after fertilization)					
Stages									
6	8	7	6	6	5	–	–	–	–
14	37	34	20	19	16	–	51	36	24
22	97	69	44	36	–	–	118	75	40
29	116	97	73	59	–	–	190	99	65
35	230	165	112	84	–	–	380	164	90
36	311	186	131	98	–	–	430	215	105
40	544	380	279	206	–	–	–	–	–
44	708	563	469	371	–	–	1316	524	308
				Proportions of normally developing embryos (% in samples).					
6–14	95.1 (0.8)*	97.3 (1.4)	95.1 (1.0)	93.8 (0.9)	9.9 (4.3)	0	86.7	95.2	74.4
22–36	87.6 (1.5)	88.6 (2.2)	83.6 (1.9)	49.1 (3.2)	0	0	67.0	74.9	47.4

* Numbers in parentheses are standard errors.

Table 2. Relationship between the timing of embryonic stages (Y-hours after fertilization) and incubation temperature (T) for lake and white sturgeon ($Y = ae^{bT}$).

Stages	White sturgeon		Lake sturgeon	
	a	b	a	b
6	11.33	−0.034	–	–
14	97.18	−0.084	109.00	−0.075
22	334.60	−0.114	358.43	−0.108
29	275.56	−0.077	534.39	−0.107
35	800.13	−0.114	1541.47	−0.144
36	1185.03	−0.127	1767.87	−0.141
40	1744.71	−0.107	–	–
44	1535.62	−0.071	5269.51	−0.145

Discussion

These results should be helpful in defining proper temperature conditions required for successful egg incubation and larval rearing of white and lake sturgeons. Embryos of both species exhibit similar temperature requirements for their normal development and survival. Successful egg incubation is possible within the temperature range 10° C to 18° C, but best results (highest survival and uniform hatching appear to be expected within the relatively narrow range of 14° to 16° C. Temperatures of 18° to 20° C may cause substantial mortalities during the sensitive embryonic stages, and temperatures above 20° C are clearly lethal, at least for the embryos of white sturgeon. Lower temperatures (10° and 11° C) exert an insignificant effect on embryonic mortality, and sublethal low temperatures are, apparently, below 10° C for both species. However, the application of temperatures below 14° C greatly

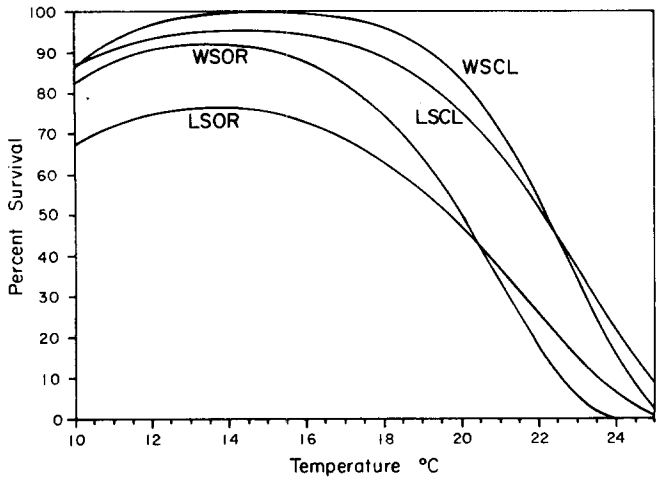

Fig. 3. The relationships between the proportions of normally developing embryos (survival) and incubation temperature: WSCL and WSOR – white sturgeon embryos during cleavage and gastrulation (stages 6–14), and organogenesis and hatching (22–36); LSCL and LSOR – lake sturgeon embryos at similar stages.

extended duration of incubation and hatching time in both species. This is undesirable in normal hatchery practice, due to increasing risk of higher mortality caused by fungal growth, a critical factor determining production of juveniles in sturgeon culture.

Our preliminary data show that embryos of white and lake sturgeon exhibit substantial similarity in their adaptations to environmental temperature. The overall effect of water temperature on the rates of development was quite similar in these allopatric species, although the lake sturgeon exhibited slower development at comparable temperatures. Both species had identical range of temperatures favorable for their survival. Experimental data are in agreement with field observations on spawning of these species. Harkness & Dymond (1961) indicated a temperature range 14–16° C as optimal for spawning of lake sturgeon. Kohlhorst (1976) observed the peak of white sturgeon spawning in the Sacramento River at a temperature of 14.4° C.

Reproduction in fishes is seasonal and their embryos and larvae exhibit species-specific and relatively narrow optimal temperature ranges (Blaxter 1969, Alderdice & Forrester 1971). Given the wide geographic range of the sturgeon species, we would expect substantial adaptive radiation in spawning temperature. Data for Eurasian species, reviewed by Detlaf et al. (1981), and data of this study appear to show the opposite, i.e. all sturgeon species studied appear to be conservative in their adaptations to spawning temperature. Sterlet, *Acipenser ruthenus*, beluga, *Huso huso*, Russian sturgeon, *Acipenser güldenstädti* and even Siberian sturgeon from the Lena River, *Acipenser baeri*, exhibit the same range of spawning temperatures of 10° C to 18° C (Schmidtov 1939, Detlaf & Ginzburg 1954, Detlaf 1970, Igumnova 1975, Nikolskaya & Sytina, 1978). The only exception is, perhaps stellate sturgeon, *Acipenser stellatus*, adapted to spawn in slightly warmer water, 15 to 25° C. Detlaf and co-workers provided experimental evidence that upper tolerance limits for embryos in all species, except for sevrjuga, is 20° C. These researchers carefully state that separation between sturgeon species by their adaptations to reproduction at different temperatures has not yet evolved (Detlaf et al. 1981).

The curves on Figure 4 show the relationships between duration of embryonic development (to hatching stage 35) and incubation temperature in five sturgeon species. Equations for the beluga, Russian and stellate sturgeon were computed from published data (Detlaf et al. 1981). The curves for white sturgeon, Russian sturgeon and stellate

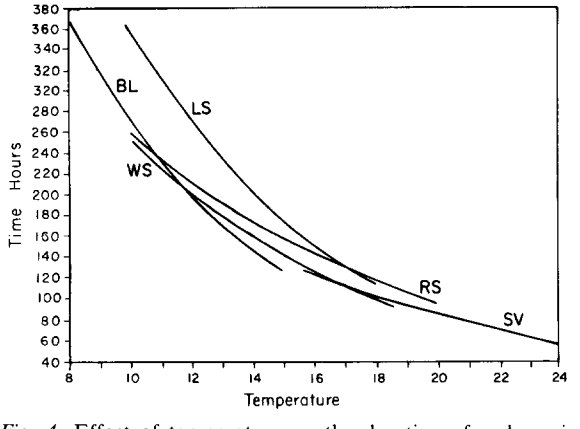

Fig. 4. Effect of temperature on the duration of embryonic development (to hatching, stage 35) in five sturgeon species: LS – lake sturgeon; WS – white sturgeon; RS – Russian sturgeon; BL – beluga; SV – stellate sturgeon. Data for RS, BL and SV are from Detlaf et al. (1981).

sturgeon are practically identical. Those for beluga and lake sturgeon have similar slopes but differ in elevation.

Acipenserids differ from modern teleosts in their possession of holoblastic cleavage and the morphogenetic processes governing their organogenesis (Detlaf & Ginzburg 1954). Ballard & Ginzburg (1980) pointed out the extreme similarity in major patterns of embryonic development existing among all sturgeon species, as a result of conservative holoblastic 'style' of early development. The effect of temperature on rate of mitosis during the early cleavage was found to be similar in several sturgeon species and was used as a universal 'dimensionless' characteristic to express the timing of early development (Detlaf & Detlaf 1960, Detlaf et al. 1981). It is possible that all sturgeon species, being highly conservative in their embryogenesis, respond with grossly similar metabolic processes to fluctuating incubation temperatures. Their embryos and larvae appear to tolerate a wide range of temperature during the reproductive seasons, roughly between 10 and 20°C in all species, although the optimal ranges may differ in different species and, possibly, in different stocks of one species.

Acknowledgements

Kenneth E. Beer (The Fishery, Sacramento) made this work possible by donating fertilized white sturgeon eggs for our study. We also thank the Department of Natural Resources, Wisconsin, and the California Department of Fish and Game for their continuous support of our research on sturgeon. This work was funded [in part] by the University of Wisconsin Sea Grant Institute under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and from the State of Wisconsin. Federal grant NA80AA-D-00086, project R/LR-26.

References cited

Alderdice, D.F. & C.R. Forrester. 1971. Effect of salinity and temperature on embryonic development of petrale sole *Eopsetta jordani*. J. Fish. Res. Board Can. 28: 727–744.

Ballard, W.W. & A.S. Ginzburg. 1980. Morphogenetic movements of acipenserid embryos. J. Exp. Zool. 213: 69–103.

Ballard, W.W. & R.G. Needham. 1964. Normal embryonic stages of *Polyodon spathula* (Walbaum). J. Morph. 114: 465–478.

Blaxter, J.H.S. 1969. Development: eggs and larvae. pp. 177–252. *In:* W.S. Hoar & D.J. Randall (ed.) Fish Physiology, Vol. 3, Academic Press, New York.

Beer, K.E. 1980. Embryonic and larval development of *Acipenser transmontanus*. M.S. Thesis, University of California-Davis, Davis. 93 pp.

Binkowski, F.P. & D.G. Czeskleba. 1980. Methods and techniques for collecting and culturing lake sturgeon eggs and larvae. Abstract of paper presented in the annual meeting of The World Mariculture Society, New Orleans.

Dean, B. 1895. The early development of gar-pike and sturgeon. J. Morph. 11: 1–62.

Detlaf, T.A. 1970. The effect of environmental temperature during the oocyte maturation and ovulation on quality of sturgeon eggs obtained in the hatchery. Trudy Inst. Osetr. Khoz. 2: 112–126. (In Russian).

Detlaf, T.A. & A.A. Detlaf. 1960. On the dimensionless characteristic of the duration of embryonic development . Dokl. Akad. Nauk. SSSR 134: 199–202. (In Russian).

Detlaf, T.A. & T.A. Ginzburg. 1954. Embryonic development in sturgeon, in connection with problems of artificial propagation. Nauka Publishers, Moscow. 204 pp.

Detlaf, T.A., T.A. Ginzburg & O.I. Shmal'gauzen. 1981. Development of sturgeon: egg maturation, fertilization, embryonic and prelarval development. Nauka Publishers, Moscow. 224 pp.

Doroshov, S.E., W.H. Clark, P.B. Lutes, R.L. Swallow, K.E. Beer, A.B. McGuire & M.D. Cochran. 1983. Artificial propagation of white sturgeon *Acipenser transmontanus* (Richardson). Aquaculture 32: 93–104.

Harkness, W.J.K. & J.R. Dymond. 1961. The lake sturgeon: the history and problems of conservation. Fish and Wildlife Branch, Ontario Dept. of Land and Forest, Toronto. 121 pp.

Igumnova, L.V. 1975. Chronological patterns of embryonic development of beluga. The Soviet J. of Dev. Biol. 6: 38–43.

Kohlhorst, D.W. 1976. Sturgeon spawning in the Sacramento River in 1963 as determined by distribution of larvae. Calif. Fish and Game 62: 32–40.

Miller, L.W. 1972. White sturgeon population characteristics in the Sacramento-San Joaquin estuary as measured by tagging. Calif. Fish and Game 58: 94–101.

Nikol'skaya, N.G. & L.A. Sytina. 1978. A comparative analysis of constant temperatures on the embryonic development of four species of sturgeon. J. Ichthyology 18: 86–98.

Schmidtov, A.I. 1939. The sterlet *Acipenser ruthenus* L. Uchenie Zapiski, Kazanaski Gosud. Univ. Zoologia 99 (6/78): 1–279.

Received 15.5.1984 *Accepted 18.3.1985*