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## Experiments on Tolerance of Incubating Walleye Eggs to Temperature Fluctuations<sup>a</sup>

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*Abstract.*—Eggs of walleyes *Stizostedion vitreum* incubating under hatchery conditions were subjected to one of five temperature fluctuations compared to normal rearing temperatures: -8.8°C on days 3-6, -9.3°C on days 7-10, +13.6°C on days 7-8, +20.2°C on days 7-8, or -9.4°C followed by +21.1°C on days 7-10. The maximum rate of temperature change was 2.5°C/hr. We found that eye-up rates remained at approximately 70% for all control and test lots. Swim-up rates of approximately 90% for the control and the first three temperature variations were reduced to 42% and 13.6% for the last two temperature variations (>20°C). We conclude that temperature fluctuations great enough to directly affect incubating walleye eggs are unlikely to occur in hatcheries or on natural spawning grounds.

The spawning success and subsequent recruitment of young walleye *Stizostedion vitreum* to natural populations is irregular throughout North America (reviewed by Koonce et al. 1977). Walleyes initiate spawning in early spring when water temperatures rise to approximately 8°C and may continue spawning for 1-3 weeks (Colby et al. 1979). The length of the incubation period is temperature dependent and typically lasts 2-3 weeks at prevailing cool temperatures (Colby et al. 1979). Many small, unprotected eggs are deposited and their

hatching success tends to be low. A hatching rate of only 60-70% is normal for the State of Michigan production facility at Mattawan (J. Copeland), and a hatching rate of 51% was reported from an Illinois hatchery (Heidinger et al. 1997). Hatching rates in the wild can be much lower (Johnson 1961). High egg mortality may occur within the first 6 h due to poor viability or lack of fertilization (Heidinger et al. 1997).

Survival of eggs and fry in the wild has been linked to weather conditions and availability of

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<sup>b</sup>Retired

food for fry (Johnson 1961; Hokanson 1977; Koonce et al. 1977; Colby et al. 1979). Generally, steadily rising temperatures and calm lake conditions during incubation favor good hatching success and strong year classes. Fluctuating temperatures during incubation are sometimes associated with weak year-classes. In Jewett Lake, Michigan, year-class failures were more likely when daily air temperature fluctuations during the egg incubation period exceeded 27.8°C, which was approximately equivalent to a 7°C daily fluctuation in water temperature (Schneider 1995). The water temperature of Jewett Lake varies more than that of most lakes in the region on sunny and calm days because it is very small, shallow, and protected from the wind, and has dark substrate.

In laboratory studies of eggs and fry, temperature ranges for optimum survival have been fairly wide: 6-12°C during fertilization, 9-15°C during egg incubation, and 15-21°C from hatching to yolk sac absorption (Koenst and Smith 1976). Four additional reports (based on controlled experiments) provided some additional information on the tolerance of walleye eggs to temperature. Hokanson (1977) reported embryo lower and upper TL50s (the temperatures lethal to 50% of the test animals) to be 6.0°C and 19.1°C respectively when rearing occurred under constant temperatures. Allbaugh and Manz (1964) reported that a change in temperature during organogenesis from 11.7 to 30.6°C and back to 11.7°C ( $\pm 19^\circ\text{C}$ ) over 2 h caused neither "excessive mortality nor any immediate adverse effect" in seven jars of eggs and only a 50% mortality in the eighth jar. Zitzow (1991) successfully incubated walleye eggs by maintaining them at 12.2-15°C for the first 3 d, reducing the temperature to 5.6-7.2°C over 8 h and holding it constant for 24 d, increasing it to about 18.3°C over 8 h, and completing incubation at 16.7-20.5°C. Thus, a 7°C decline and a 12°C increase, each over 8 h, were not harmful. Malison et al. (2001), during attempts to create polyploids, reported some mortality of walleye eggs 1-5 min old when temperature was abruptly increased from 11°C to 29-32°C. These results suggest walleye eggs are quite tolerant of temperature changes as great as  $\pm 19^\circ\text{C}$ . For fry in the yolk sac stage, Clapp et al. (1997) recommended that stocking

not occur when receiving water was either above 20°C or more than 6°C higher than transport water. However, Clapp et al. (1997) found that fry tolerated temperature changes of 1.5°C/min, a relatively high rate compared to changes likely to occur in natural spawning areas.

The purpose of the following experiments were to evaluate walleye development rates to the eye-up and swim-up stages after exposure to three levels of temperature fluctuations under controlled hatchery conditions. Such information could be used to evaluate limitations on the spawning success of wild walleye populations as well as potential constraints on hatchery operations.

## Methods

Experiments were conducted at the Wolf Lake State Fish Hatchery in Mattawan, Michigan, the state's primary walleye culture facility. Standard egg collection and hatchery practices were followed except for prescribed temperature fluctuations. Adult walleyes were captured from the Muskegon River by electrofishing on April 8, 1996, and held in a pond until ripe; then their eggs were stripped and fertilized at 6.8°C. Bentonite was added to prevent egg clumping. Eggs were transported to the hatchery where temperature was maintained constant at 10-11°C by groundwater. Well-mixed samples of eggs were distributed among 14 incubation jars at a density of 1 L of eggs (approximately 139,600 eggs) per jar. All jars were treated with formalin for fungus control for the first 8 d of incubation at a dosage of 1:600 for 15 min. No disease problems were encountered during culture and dead eggs were not culled until after the eye-up stage.

Of the 14 jars, 4 were controls incubated at normal temperatures (10.5°C, raised to 15°C near hatching) and 10 received one of five experimental treatments. Experimental jars were gradually subjected to either two types of cold stress (-8.8°C on days 3-6 or -9.3°C on days 7-10), two types of heat stress (+13.6°C or +20.2°C on days 7-10), or a combination cold and heat stress (-9.4°C followed by +21.1°C over days 7-10), as described in Table 1. Eggs in the first four treatments were returned to the normal

temperature sequence; eggs in the fifth group were hatched at 22.2°C. The maximum rate of temperature change was 2.5°C/hr. Egg survival rates were measured at eye-up, which occurred about 7 d after fertilization. After that, survivors in replicate jars were combined, except for treatment 5. Survival rates were determined again at swim-up, which occurred about 3 d after hatching, and the number of fry per cubic centimeter was determined. Chi-square tests and decomposition methods given by Snedecor and Cochran (1989:197) were used to evaluate statistical significance ( $P < 0.05$ ).

### Results

Temperature treatments had no effect on eye-up rate but a significant effect on swim-up rate (Table 2). Eggs in all control and experimental jars eyed-up at rates of 67-70%, the same as eggs incubated in hatching jars for normal production elsewhere in the hatchery. Swim-up rates were significantly different across all groups ( $\chi^2 = 93.1$ ,  $df = 4$ ). Swim-up rates were similar (90-94%) for control eggs and those exposed to thermal fluctuations as great as 13.6°C. However, swim-up rates were 42% for stresses of +20.2°C and 11-16% for stresses of  $\pm 21.2^\circ\text{C}$ . Those two extreme treatments contributed nearly all of the significance to the chi-square test. We also observed higher rates of deformity in the high temperature-stressed groups as well as a tendency for there to be slightly fewer fry per cubic centimeter (Table 2).

### Discussion

Developing walleye eggs exhibit a remarkable tolerance to temperature fluctuations. No effect in swim-up survival was evident at a heat stress of +13.6°C, and some swim-up fry were produced at a combined cold and heat stress of  $\pm 21.2^\circ\text{C}$ . This finding supports prior hatchery observations in which temperature fluctuations as great as  $\pm 19^\circ\text{C}$  had only small effects on incubation success (Allbaugh and Manz 1964; Zitzow 1991). Egg hatching success in our experiment was better than anticipated from Hokanson's (1977) report

that constant incubation temperatures of less than 6.0°C or more than 19.1°C would be detrimental; however, those values were only briefly exceeded during our experiment. In experimental attempts to induce triploidy, walleye eggs were also resistant to very abrupt heat shocks applied 1-5 min after fertilization and lasting 10-25 min (Malison et al. 2001). Survival was moderately reduced by an increase from 11°C to 28°C and substantially reduced by increases from 11°C to 30-32°C.

These results have implications for cultural operations as well as for the walleye recruitment process in nature. In hatcheries, temperature need not be rigidly controlled. In nature, walleye eggs are sufficiently tolerant that temperature fluctuations are not likely to cause direct mortality. This is a fitting adaptation for a species that spawns in early spring. Fluctuations in air temperature and solar heating can be considerable in early spring, but fluctuations in water temperature are buffered by the cool water mass. On walleye spawning grounds in lakes and rivers, temperature fluctuations high enough to reduce hatching success (between 14°C and 19°C) are very unlikely to occur.

However, six other temperature-related factors may affect walleye recruitment. First, stressed eggs may produce stressed fry that will not perform well after swim-up, the last stage we monitored. In other species of fish, there is evidence that reduced fitness may appear at a later stage (von Westernhagen 1988). Second, the optimum of 15-21°C for fry (Keast and Smith 1976) may not occur at the required time in relatively cold habitats such as ground water-fed rivers or very large northern lakes. Third, fry may not adapt well from endogenous to exogenous feeding, a vulnerable stage that is indirectly temperature dependent because it requires a coincidental pulse in zooplankton of the proper density, species, and size (Li and Mathias 1982; Johnston and Mathias 1993, 1994; Mayer and Wahl 1997; Jonas and Wahl 1998). Fourth, high temperatures can stimulate the growth of filamentous algae and fungi that can smother eggs (Colby et al. 1979). Fifth, cool temperatures prolong the incubation period and increase the risk of losses to storms, predation, or disease (Colby et al. 1979). These factors seem more likely to affect recruitment

than direct lethal effects of temperature on incubation success.

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Table 1.–Control and experimental treatments applied to incubating walleye eggs. The control treatment was applied to four incubation jars, the other treatments to two jars each; each jar contained approximately 139,6000 eggs.

Treatment	Description of temperature changes
Control	Held at 10-11°C on days 1-15; on day 16 raised to 15°C and held until hatched.
Cold: -8.8°C on day 3	On day 3 lowered to 1.6°C in 5 h and held; on day 6 returned to 10.5°C and held; on day 18 raised to 15°C and held until hatched.
Cold: -9.3°C on day 7	On day 7 lowered to 1.2°C over 8 h and held; on day 10 returned to 10.6°C and held; on day 18 raised to 15°C and held until hatched.
Heat: +13.6°C on day7	On day 7 raised to 24.2°C over 12 h and returned to 10.5°C over 8 h and held; on day 15 raised to 15°C and held until hatched.
Heat: +20.2°C on day 7	On day 7 raised to 30.7°C over 12 h and returned to 10.5°C over 8 h and held; on day 15 raised to 15°C and held until hatched.
Cold and heat: ±21.1°C on days 7-10	On day 7 lowered to 1.1°C over 14 h; on day 9 raised to 22.2C over 2 d and held until hatched.

Table 2.—Days to hatch, percent eye-up and swim-up, and number of fry/cm<sup>3</sup> for walleye eggs subjected to cold and heat shocks during incubation. The control treatment was applied to four incubation jars, the other treatments to two jars each. Mean values for the control were as follows: hatch = 16 d; eye-up = 69.8%; swim-up = 91.9%; and fry/cm<sup>3</sup> = 200.

Treatment	Temperature sequence (°C)	Hatch (d)	Eye-up (%)	Swim-up (%)	Fry/cm <sup>3</sup>
Control	10-11, 15	16	72.0		
		16	69.9	94.0	207
		16	69.1		
		16	68.3	89.8	192
Cold: -8.8°C on day 3	10-11, 1.6, 10.5, 15	18	62.8		
		18	72.2	92.7	156
Cold: -9.3°C on day 7	10-11, 1.2, 10.6, 15	18	67.8		
		18	69.1	90.6	170
Heat: +13.6°C on day 7	10-11, 24.2, 10.5, 15	15	68.4		
		15	65.7	89.6	185
Heat: +20.2°C on day 7	10-11, 30.7, 10.5, 15	15	66.6		
		15	67.3	42.0	137
Cold and heat: ±21.1°C on days 7-10	10-11, 1.1, 22.2	12	69.0	16.1	156
		12	69.0	11.2	135

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