

Intensive Culture of Walleye Larvae Produced Out of Season and during Regular Season Spawning

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Abstract.—The performance of larval walleyes *Stizostedion vitreum* that were produced out of season was compared with larvae produced during a regular season, using intensive culture methods. Wild-captured walleye adults were held in an earthen pond beginning in late November and then gradually transferred to higher water temperatures before ovulation was hormonally induced in February. A second batch of walleye larvae was obtained from a hatchery operation in April and otherwise reared in similar conditions. The two experiments were conducted to evaluate survival and growth of walleye larvae reared initially on live nauplii of brine shrimp *Artemia salina* and then gradually weaned to commercial diets. Larvae were stocked at 20 fish/L and raised in large (800-L) triplicate tanks provided with turbid water (40–50 nephelometric turbidity units) and surface sprays. Mean final weight of fish after 32 d of rearing from the first (February; 100.5 ± 17.5 mg) and second (April; 111.2 ± 18.0 mg) batch did not differ significantly, whereas survival was $6 \pm 1.9\%$ for juveniles produced out of season and $47 \pm 1.6\%$ for those produced during the regular season. The lower survival rate obtained with out-of-season juveniles can be attributed to somewhat altered egg quality, slightly different dietary regimes, and other physical variables. As these variables are optimized, the ability to sequentially wean two cohorts of juvenile walleyes to commercial diets in the same facility, while producing a 5-week growth advantage to one cohort, may become of significance to aquaculture.

Culture procedures for rearing larval and juvenile walleyes *Stizostedion vitreum* include out-of-season spawning, as early as late January or 2–

3 months before the regular season (Malison et al. 1998), and intensive, high density tank-rearing of juveniles (Moore et al. 1994a; Bristow et al. 1996). Progress made in recent years demonstrates that hormonal injections can induce early spermiation and ovulation in walleyes (Malison et al. 1994). Relatively simple photothermal manipulation and gonadotropin or releasing-factor injections allow for successful ovulation of walleyes and high survival of their embryos until the eyed egg stage (Malison et al. 1998). Survival of embryos varies, depending on the hormonal treatment used. Previous studies have not examined survival and growth of out-of-season walleyes.

Major advances have been made in recent years in the intensive culture of walleye larvae with changes in tank design, use of surface water sprays that improve gas bladder inflation (Moore et al. 1994b), and use of turbid water that reduces cannibalism (Rieger and Summerfelt 1997). Moore et al. (1994a) found that walleye juvenile growth was not affected by different densities (20–60 fish/L) or tank size (278–679 L). Currently, successful culture of walleyes includes utilization of formulated feeds (Barrows et al. 1993), in addition to previous techniques based solely on live feeds (Krise and Meade 1986).

The size of walleye juveniles at the time of stocking in summer is an important predictor of potential fingerling survival over the winter and ultimately recruitment. Therefore, from the fisheries management perspective it is important to produce the largest possible fish sizes at stocking. Because out-of-season production gives an addi-

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tional 2 months of growing before stocking time, successful development of this culture technique would greatly enhance recruitment potential. The objectives of the research presented here were to evaluate the suitability of walleye larvae produced out of season by comparing them to fish produced in the regular season and to simulate rearing systems used commercially (i.e., large, 800-L production tanks).

Methods

Studies were conducted at Piketon Research and Extension Center, The Ohio State University (Piketon, Ohio). Two experiments (spawnings) were conducted. First, brood walleyes, transported from Lake Erie, were acclimated in a pond from November to December 1997 before being transferred indoors in January 1998 for induced ovulation. Second, walleye gametes were collected in April 1998 from naturally spawning fish in C. J. Brown Reservoir, Ohio; these fish are also of Lake Erie origin. The growth and survival of the two batches of walleye larvae were compared under similar intensive culture conditions.

Spawning and production.—In the out-of-season experiment, four walleye females were used and each received two injections (January 27 and 28) of human chorionic gonadotropin (HCG, Sigma Chemical Co., St. Louis, Missouri). The primary and resolving injections were 150 IU/kg and 500 IU/kg, respectively, given at an interval of 35 h. Eggs, collected by stripping on February 2 and 4 from two of the females, were immediately fertilized with fresh walleye sperm. Male spermiation was stimulated by temperature increase to 9°C associated with the transfer from the pond to indoor tanks. Eggs were incubated in 6-L McDonald jars with continuous water flow (8–9°C). Formalin treatment was applied daily to the eggs to prevent fungal infections (500 mg/L for 30 min). On February 19, when embryos started to hatch, water temperature was increased to 12°C to synchronize hatching. Most of the embryos hatched during February 25–26 and were transferred to a holding tank by overflow. On March 6 larvae were placed in 800-L circular culture tanks. The stocking density was 16,000 larvae/tank (20 fish/L) in three replicates; we enumerated larvae with an electronic larval fish counter (Jensorter, Inc., model FC 2).

In the regular season experiment (experiment 2; fish from C. J. Brown Reservoir), eggs were fertilized immediately after spawning and transported to St. Mary's State Fish Hatchery, Ohio. The in-

cubation temperature was maintained at 11°C, and the eggs were treated with the formalin (as above) to prevent fungal infection. After hatching, 6–7-d-old walleye larvae (offspring of several females whose eggs were incubated together) were transported to Piketon Research and Extension Center, Ohio, and were immediately placed into 800-L tanks at 16,000 larvae/tank in three replicates.

Experimental design.—The same rearing systems (800-L cylindrical tanks as described by Moore et al. 1994b) were used in both experiments. The water flow was set at 4–5 L/min with the two surface spray points supplying an additional 750 mL/min each. The central standpipe in each tank was covered with 500- μ m mesh screen. Turbidity in the culture tanks was maintained at a level of 40–50 nephelometric turbidity units (NTU; Turbidimeter, 2100 P, Hatch, Loveland, Colorado) by a constant supply of clay solution to the system injected with a peristaltic pump (Masterflex, model 7021–24, Cole Parmer Instr., Vernon Hills, Illinois) through inlet pipes. Water temperature was maintained at approximately 16°C throughout the experiment. Photoperiod was 12 h daylight with light intensity at 150 lx and 12 h of dark.

The San Francisco strain of brine shrimp *Artemia salina* (Argent Chemical Laboratory, Redmond, Washington) was used in this study; cysts were decapsulated before incubation (Campton and Busack 1989) and incubated (5 g/L) in 30 g/L salinity at 27°C under continuous aeration in 40-L conical tanks. After 24 h of incubation, freshly hatched nauplii were washed with salt water and kept in a 100-L plastic barrel with aeration. Brine shrimp were continuously supplied to culture tanks using a peristaltic pump, and the amounts fed to walleyes was adjusted daily. Weaning of the walleyes spawned out of season to a dry diet started after 10 d of exclusive feeding on brine shrimp nauplii. The brine shrimp diet was gradually replaced by Biokyowa feed (Kyowa, Japan; mixture of 400B and 700B). After 2 weeks of cofeeding, brine shrimp were completely eliminated from the diet and only the Biokyowa dry diet was fed to the fish during the last 10 d of the experiment. The feeding schedule of the walleyes spawned during the regular season was altered; the dry diet was introduced after 20 d of culture but the supply of brine shrimp was continued at a low level to encourage feeding behavior.

Daily measurements of temperature, turbidity, and dissolved oxygen were recorded. A siphon was used daily to remove accumulations of clay, dead

brine shrimp, and fish larvae from the bottom of each tank. Standpipes were removed and cleaned every day, and tank walls were cleaned every few days. In the second experiment only, daily fish mortality was estimated by counting dead larvae in the solid wastes removed from each tank.

Samples of 10–20 viable larvae from each tank were collected every other day. Presence of food in the gut and swim bladder inflation were recorded. These same larvae were measured for standard length and wet weight and dried in the laboratory oven at 60°C for 2 d and weighed again (dry weight). Survival of walleyes in each experiment was determined by counting all the viable fish remaining. Specific growth rates and condition factors (Dabrowski et al. 1992) were calculated at the end of each experiment. The average values of these parameters were compared using Student's *t*-test (SPSS 7.5 for Windows, SPSS, Inc., Chicago, Illinois).

Results

There were significant fluctuations in environmental conditions during the first 2 weeks of experiment 1 in March (Figure 1). Adjustments made during operations resulted in water temperature and turbidity stabilization thereafter. In the second experiment environmental conditions (temperature and turbidity) were stable.

Growth and condition factors of fish cultured in the two experiments were not significantly different (Table 1). However, transition to a solely dry diet affected fish growth by a cessation of growth for several days in experiment 1 (Figure 1, weight panel). Complete swim bladder inflation by walleyes was observed at similar fish sizes in experiment 1 (9.40 ± 0.19 mm and 6.24 ± 0.97 mg) and experiment 2 (10.13 ± 0.6 mm and 6.74 ± 0.45 mg). However, final survival rates of walleyes were significantly different in experiment 1 versus 2 ($6.0 \pm 0.2\%$ versus $47.0 \pm 1.6\%$). Although the environmental conditions were stable in the second experiment, particularly high mortality was observed immediately following stocking larvae to the tanks, and 1 week after stocking, mortality increased again when incidences of cannibalism were observed (Figure 2). Considerable discrepancy (53%) exists between final survival and total evaluated mortality based on the number of collected dead fish. Underestimated daily mortality can be the consequence of cannibalism (up to 21%; Rieger and Summerfelt 1997) and deterioration of larvae bodies before tank cleaning.

Feeding rates with dry diets were estimated in

the second trial, based on average fish weight and daily recorded survival (Moore 1996). Because of discrepancy in mortality estimation, feeding rates on formulated and live diets were larger than projected in the regular season experiment. Nevertheless, fish were fed to satiation in both experiments, based on the amount of feed removed after daily cleaning.

Discussion

Survival was substantially greater for walleye larvae produced from the regular season than the off season, although no significant differences were found in fish sizes and condition factors between the two groups after about 30 d. The discrepancy in survival rates could have been affected by a number of physical and biological factors. Low water temperature is known to be a critical limiting factor in intensive culture of walleye larvae. In both of our experiments the average water temperature was the same (16°C); however, its fluctuation at the beginning of experiment 1 could have negatively affected walleye survival. Similarly, unstable turbidity levels during the first week of experiment 1, especially its drop to 5 NTU on day 7 and 10 NTU on day 8 of the culture might have triggered cannibalistic behavior, thus increasing mortality.

Broodstock origin and consequently genetic and nutritional differences affect biochemical composition of walleye eggs. For instance, Czesny and Dabrowski (1998) showed that concentrations of specific fatty acids were positively correlated with embryo survival, indicating altered quality of eggs with respect to chemical composition. Johnston (1997) demonstrated that within a single population of spawning walleyes, female age positively correlated with hatching success. One can speculate that variation in egg quality can subsequently affect the quality and robustness of fish embryos and larvae after hatching. Nevertheless, findings for yellow perch *Perca flavescens* larvae (Kolkovski and Dabrowski 1998) support the survival results in this study, indicating that out-of-season fish larvae are of inferior quality. We found significantly higher incidences of deformities and early mortality in yellow perch larvae produced out of season compared with those of the regular season.

Turbid water and surface spraying minimize cannibalism and enhance swim bladder inflation (Bristow et al. 1996). As in earlier studies (Bristow and Summerfelt 1994), we observed complete swim bladder inflation, food acceptance, and no

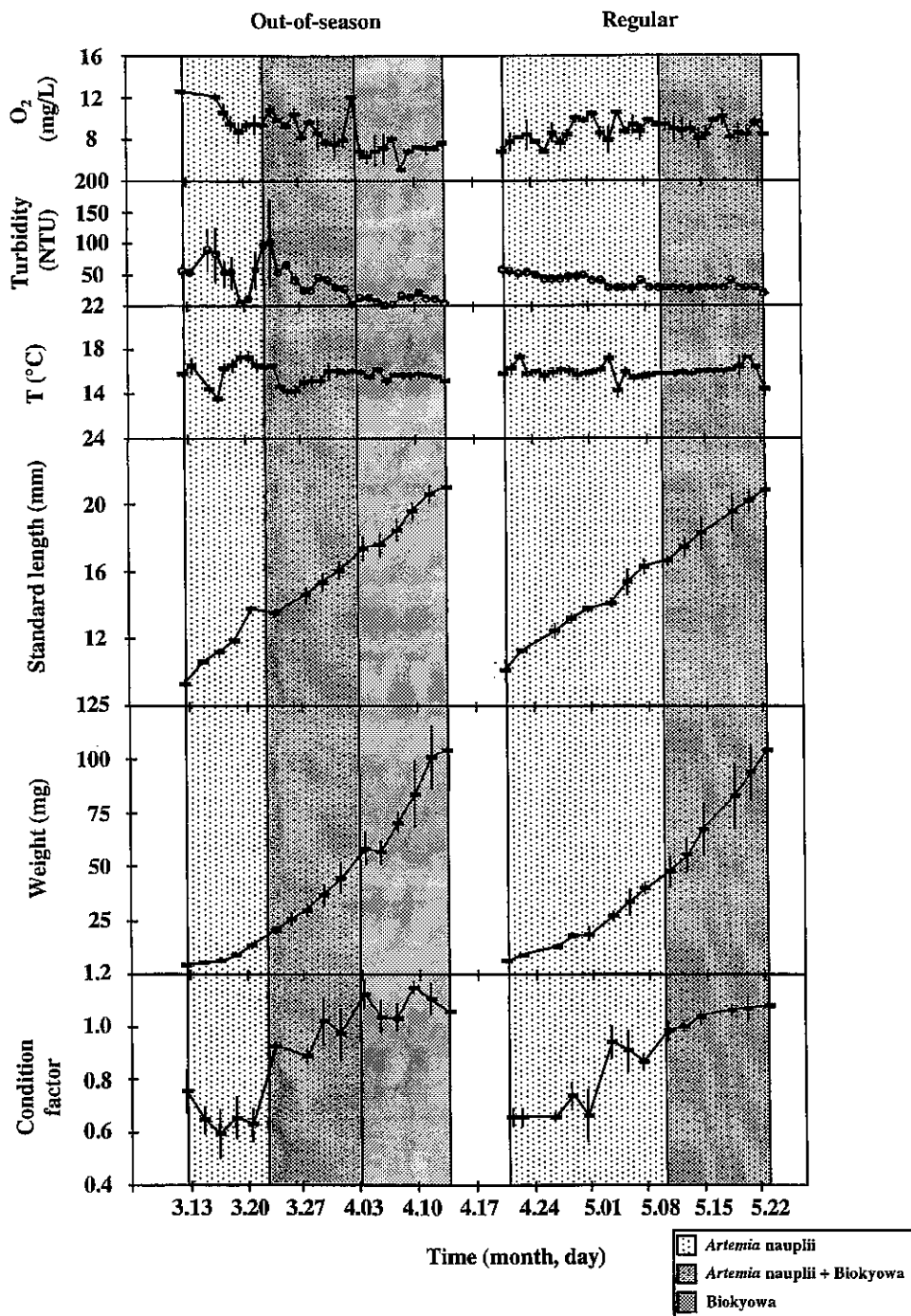


FIGURE 1.—Environmental conditions (T = temperature) in rearing tanks and rates of growth of walleye juveniles during periods of being fed live artemia nauplii, formulated food (*Biokyowa*), or both. Vertical lines represent 95% confidence intervals.

TABLE 1.—Comparison of final body size and survival of larval walleyes in two rearing experiments. Significant difference ($P < 0.05$) is marked with asterisk.

Variable	Out-of-season spawning	Regular spawning
Standard length (mm)	21.1 ± 1.1	21.8 ± 1.0
Wet weight (mg)	100.6 ± 17.5	111.2 ± 18.0
Dry weight (mg)	16.8 ± 3.0	17.7 ± 2.6
Condition factor ^a	1.1 ± 0.0	1.1 ± 0.0
Survival (%)	6.0 ± 0.2	47.0 ± 1.6*
Specific growth rate ^b (%/d)	4.4 ± 0.2	4.5 ± 0.1

^a Condition factor = $100 \times \text{final weight}/\text{length}^3$.

^b Specific growth rate = $[(\log_e \text{ final weight} - \log_e \text{ initial weight})/\text{d}] \times 100$.

“clinging” behavior. Tank size and fish density in our study were comparable to those used by Moore et al. (1994a), who also observed considerable variation in survival of walleye juveniles (8.6–74.9%) until day 21. Bacterial gill disease was identified as a cause of mortality in their experiments. Although we used a prophylactic treatment of 50 mg/L of formalin in both experiments, we noticed considerable improvement in experiment 2 (regular spawning) compared with experiment 1 (out-of-season spawning); the causal effect was not determined.

Moore et al. (1994a) found a highly significant effect of water temperature on juvenile walleye growth. With a fish density comparable to that used in our study (20 fish/L) at 13.3, 18.7, and 20.3°C, walleye juveniles attained 13.5, 19.2, and 22.3 mm, respectively, after 21 d (Moore et al. 1994a). In our study lasting 32 d at average water tem-

peratures of 16°C, final mean fish lengths were 21.1 and 21.8 mm (Table 1). Whether optimal temperatures (estimated to be in the range of 24–26°C by Hokanson and Koenst 1986) for growth of juvenile walleyes weighing about 27 g are applicable to rearing larval walleye in intensive systems has not been determined. Qin et al. (1994) reported significantly higher growth of walleyes in ponds at a substantially lower density (10–50 fish/m³) that was than in our study (0.67 and 0.32 g after 35 d).

Exclusive use of dry diets in intensive culture of walleyes has also been successful and this technique has been used for several seasons (Moore 1996; A. A. Moore, Iowa Department of Natural Resources, personal communication). Although live food was used in our study for an extended period in the regular-season experiment, survival (47%) compares favorably (9–11%) with earlier studies on walleye larvae fed dry feeds exclusively (Barrows et al. 1993). Therefore, further studies in out-of-season production should include dry feed exclusively and earlier weaning to formulated feeds.

Although physicochemical variables were not precisely controlled in the out-of-season experiment, we have shown that considerable numbers of walleyes can be grown before their regular spawning season. In our study, the sequence of two rearing periods in the same facility is also appealing, in addition to the 5-week growth advantage given to the first batch of fish. Accompanying studies with live-food enrichment and diet

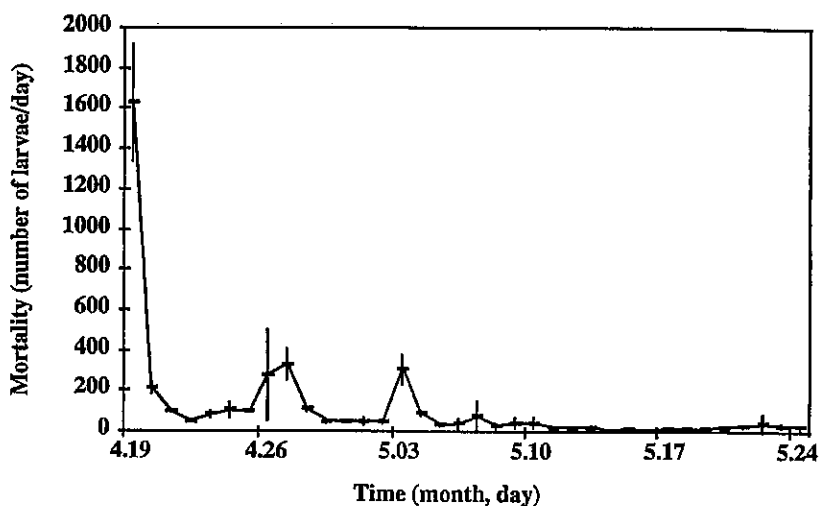


FIGURE 2.—Daily mortality rates of larval walleye following the regular-season spawning of broodstock collected in C. J. Brown Reservoir.

formulation, which included supplements of essential fatty acids and vitamins (Czesny et al. 1999; Kolkovski et al. 1999), point out the need for further improvements in the rearing process of larval walleyes.

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