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ABSTRACT

Texas Parks and Wildlife Department fish hatchery staffs have used a standard index of 275 fry/g to estimate numbers of Florida largemouth bass (FLMB) *Micropterus salmoides floridanus* fry since 1992. However, the accuracy of these fry estimates has not been verified. We evaluated this index for accuracy using a Jensorter fry counter. Because the Jensorter fry counter had not been tested for FLMB fry enumeration, we first tested it for accuracy and precision by comparing it to hand counting. Fry (5-11 d old) were produced from March to June in 25 incubation troughs. For the Jensorter accuracy verification, a sample of fry (~100 fry) was taken from each incubation trough and hand counted, followed by anesthetization of fry and re-counting using the Jensorter fry counter. Percent accuracy and precision were calculated from the data. For verification of the standard index, three samples of fry (~100 fry each) were taken from each trough and weighed. Fry in each weighed sample were anesthetized and counted using the Jensorter. Approximately 40 fry from each weighed sample were individually measured for total lengths. A gravimetric index (fry/g) was calculated for each trough of fish. These indices were divided into groups (fish age group: <7 d, 7-9 d, and ≥ 10 d postspawn; production cycle: early and late spring; and entire study period: data for March - June) and compared to the standard index. The Jensorter was 98% accurate compared to hand counting, and precision was very good (2.2%). The mean index (fry/g) was 289 for all fry for the entire study period; 284 for all fry for early spring; 300 for all fry for late-spring; 315 for <7-d-old fry and 287 for 7-9-d-old fry produced in late spring; and 310 for ≥10-d-old fry produced in early spring. These indices were statistically greater than the standard index ($P < 0.05$). Conversely, the index for the 7-9-d fry was 274 fry/g for early spring and 277 fry/g for the entire study period, and each did not statistically differ from the standard index ($P > 0.05$). The index and fish length results suggest that incubation water temperature, which correlated with production period, influenced fish size and thereby the gravimetric index. Our results suggest that the standard index is appropriate for enumerating 7- to 9-d-old fry produced in early spring or during an entire production period. Further, the results suggest that a gravimetric index should be defined by age of fry and incubation temperature.

INTRODUCTION

Proper fish pond management decisions, reliable fish production data, and defensible fish culture research results depend, at least partly, on reliable fish population estimates. Furthermore, accurate and quick methods of quantifying fish populations are essential for effective execution of research studies and proper and efficient operation of fish production facilities. Unfortunately, enumeration of large numbers of larval fish or fry is difficult because, unlike fingerlings and adults, sampling of these fish for weight measurements without significant losses to mortality is often impossible, and weight tables (Piper et al. 1982) are unavailable. Current methods of enumerating larval fish or fry include volumetric and gravimetric options (Piper et. al 1982; Harrell et al. 1990). These methods involve counting a few samples of fish from a population to determine an average number/g or number/mL and multiplying the average value by the total weight or volume of the fish to obtain the total number of fish. To eliminate the determination of the average number/g or number/mL for each batch of fish and save time, culturists use indices. These indices are the average numbers/g or numbers/mL that are determined once and used year after year as standard indices.

Standard indices, however, may be unreliable if used indiscriminately because weight and length of fish are influenced by fish age and condition. These growth variables also are influenced by water quality variables, particularly temperature which appears to be the most important physical factor that controls growth and development of fish (Piper et al 1982; Fry 1971; Colt and Tomasso 2001). Despite the potential problem of miscounting fry by using a standard index, several hatchery managers prefer to use such indices because they provide flexibility and efficiency, in terms of time management, in hatchery operations.

Texas Parks and Wildlife Department (TPWD) freshwater fish hatcheries use a standard index of 275 fry/g to estimate numbers of Florida largemouth bass (FLMB) fry for stocking into ponds or distribution to other hatcheries. This index was developed by conducting a series of hand counts of weighed grab samples of swim-up fry and determining a mean number of fry/g (J. Isaac, A. E. Wood Fish Hatchery, San Marcos, Texas, personnel communication). Swim-up FLMB fry are typically 7-10 d old postspawn at time of enumeration (H. Glenewinkel, A. E. Wood Fish Hatchery, San Marcos, Texas, personal communication); however, they may include older and younger fry. This happens because spatial constraints force culturists to incubate spawns of different ages (i.e., spawns collected in two or three consecutive days) together as one cohort. Despite this lumping of dissimilar-age spawns and consequently dissimilar-age fry, the current index is indiscriminately used for all bass fry enumeration because it allows several millions of fry to be quantified quickly. At TPWD fish hatcheries where this standard index is used, fingerling production in ponds has been inconsistent; percent returns have been disproportionably low or high (e.g., >100%), suggesting inaccuracies in initial fry enumeration involving the use of the standard index.

Despite the suspicion that the standard index may be contributing to the inconsistent bass fingerling production results, the index has not been evaluated for accuracy since its use was implemented in 1992. We conducted this study to verify the accuracy of the standard index using the Jensorter fry counter (model FC 2; Jensorter, LLC, Bend, Oregon). The Jensorter counter is an electronic device that has been used to enumerate fry of striped bass *Morone saxatilis* (Lemarie et al. 1997) and walleye *Stizostedion vitreum* (Kindschi and Barrows 1991) quickly but with varying degree of accuracy and precision. Kindschi and Barrows (1991) reported better results and further determined that hand count was the most accurate method of enumerating walleye fry, followed by the Jensorter, gravimetric, and volumetric methods in that order. We are unaware of any documented evidence of the accuracy of the Jensorter in enumerating FLMB fry. The objectives of this study were to 1) investigate the accuracy of the Jensorter fry counter in enumerating FLMB fry, 2) document the validity of the current standard gravimetric index of 275 fry/g for estimating numbers of FLMB fry, and 3) document the effects of water temperature and incubation period on the standard gravimetric index used by TPWD hatcheries to enumerate FLMB fry.

MATERIALS AND METHODS

Fry Production.—This study was conducted at the A. E. Wood Fish Hatchery, Hays County, Texas during a routine production of FLMB fry for fingerling culture in ponds. We spawned two year classes of FLMB broodfish separately in two production cycles: year-class 1993 in early spring (March–April) and 1994 in late spring (May-June). These broodfish year classes, created within the TPWD hatchery system from the same genetic line, were subjected to the same feed and feeding regimen (Hutson 1990) prior to spawning. Broodfish were spawned in indoor concrete raceways, and the spawns were collected for incubation daily. Spawns were incubated and the resulting fry held in indoor 2,820-L rectangular fiberglass troughs using established culture practices (Hutson 1990). Due to limited trough space, spawns collected 2-3 d apart were sometimes incubated together in the same trough to produce fry that were 2-3 d old apart but were considered as one cohort. Fry samples for the study were taken when fry were at the swim-up stage (5-11-d old postspawn).

Water Quality.—Water for FLMB spawning, egg incubation, and fry culture was from the San Marcos River, Hays County, Texas. The water reached the culture facilities (raceways and incubation system) either directly from the river or indirectly via a 3.24-ha outdoor water storage reservoir. These waters typically have stable pH, dissolved oxygen (DO), and total hardness (317mg/L as $CaCO_3$) levels; however, they are prone to seasonal changes in temperature. We managed water quality variables, especially water temperature, within ranges considered suitable for FLMB spawning and egg incubation. These suitable conditions included temperatures of 18.3–23.9ºC, DO > 4.0 mg/L, and pH 6.5–8.0 (Piper et al. 1982; Hutson 1990; Tidwell et al. 2000). The quality of the incubation water was managed by using river water only early in the season, followed by reservoir water only, and then a mixture of river and reservoir waters later in the season. Water temperature and DO concentration were monitored once daily at 1400 hours in each raceway or incubation trough that contained eggs or fry. Daily values of pH were recorded for the incoming water. These variables were measured with a Yellow Springs Instruments (YSI) model 55 dissolved oxygen meter.

Evaluation of the Jensorter Fry Counter.—Visual observation of fry actively swimming in the water column was the criterion for determining the swim-up stage of the fish and initiating fry sampling. Incubation troughs were checked for swim-up fry once daily in the afternoons. On the day the fish were considered to have reached the swim-up stage, a sample was taken from each incubation trough using a small aquarium net. A sub-sample of approximately 100 fry was taken from each sample and hand counted. These fry were then anesthetized using MS-222 (Kindschi and Barrows 1991) and re-counted using the Jensorter fry counter. The Jensorter was equipped with a plate (# 5) with eight 3.2-mm holes for water outlet and fry exit, and it received gravity-flow water from a header box. The Jensorter counts were compared to the hand counts, which served as the standard (Kindschi and Barrows 1991), to determine percent accuracy {100(Jensorter – hand)/hand}. Precision (100 * SD/mean; Steel and Torrie 1980) of the Jensorter counts also was determined.

Verification of the 275 fry/g Standard Gravimetric Index.—A total of 73 samples of swim-up fry, 51 for early spring and 22 for late spring, taken from the 25 incubation troughs were used for this phase of the study. Each fry sample was taken with a small aquarium net. Excess water was allowed to drain from the sample for about 30 s and the residual water blotted off the net using absorbent paper towels. These fry were transferred into a tarred container of water for weight measurement. Weights were measured to the nearest 0.1 g using a calibrated Mettler PE-11 electronic scale (Mettler-Toledo, Inc., Columbus, OH). Each weighed sample was anesthetized and counted using the Jensorter as described above. Then about 40 fish in a sub-sample were individually measured for total lengths to the nearest 0.1 mm using a Manostat SPI Dial Caliper (Swiss Precision Instruments, Inc., Garden Grove, CA). A gravimetric index (fry/g) was calculated for each trough of fish.

Data Analysis.—The Jensorter and hand-count data were compared using a one-sample Student's *t*-test ($\alpha = 0.05$; Ho: $\mu = 100$). Similarly, the gravimetric index data were compared with the standard gravimetric index of 275 fry/g using a one-sample Student's *t*-test ($\alpha = 0.05$; Ho: $\mu = 275$). The gravimetric index data were analyzed by the following group variables: fish age group (<7 d, 7-9 d, and ≥ 10 d postspawn), production cycle (early and late spring), incubation water temperature, and study period (data for March-June). The Systat version 9 statistical software was used to perform all analyses. Differences were considered significant at *p*-values equal to or less than 0.05.

RESULTS AND DISCUSSION

Water Quality and Fry Production.—Water temperatures were 19.0–24.7°C during the early-spring production cycle and 23.0–27.6°C during the late-spring cycle (Table 1). The pH ranged from 8.0 to 8.2, and DO concentrations were above 6.0 mg/L throughout the study. These water quality variables were suitable for FLMB spawning and fry production (Moss and Scott 1961; Dudley and Eipper 1975; Boyd and Lichtkoppler 1979; Piper et al. 1982; Coutant and DeAngelis 1983; Dupree and Huner 1984; Tidwell et al. 2000). The average weights of the females spawned during early and late spring were 2.5 and 2.0 kg, respectively. A total of 319 spawns from 180 females produced over 3 million fry in early spring whereas 90 females produced 287 spawns and over 2 million fry in late spring. The spawning periods were 23 and 33 d for the early and late spring, respectively. Production efficiencies were 1.8 spawns/female (290 fry/kg female/d) and 3.2 spawns/female (337 fry/kg female/d) for the early- and late-spring production cycles, respectively. The late spring females were more productive probably because of the smaller size, longer acclimation period, longer spawning period, or a combination of these factors (Mayes et al. 1993; Davis and Lock 1997). These results are similar to the production performances of the previous two years at the A. E. Wood Fish Hatchery.

Evaluation of the Jensorter Fry Counter.—A total of 25 fry samples, one from each of the 25 incubation troughs used for the study, were enumerated with the Jensorter fry counter. The mean count of all of the samples was 102 which was not significantly different (P = 0.182) from the hand-count value of 100. The accuracy of the Jensorter was 98% relative to the hand count and precision was 2.2%. These results agree with the 98.4% accuracy and 1.4% precision derived from the results for the same model Jensorter used to count 3-4-d old walleye *Stizostedion vitreum* (see Table 1in Kindschi and Barrows 1991). Further, our level of accuracy was the same as the maximum (98%) reported in the Jensorter specifications by the manufacturer and similar to the 97% for all tests reported by Kindschi and Barrows (1991). Thus, we conclude that the Jensorter fry counter was accurate and precise for quantifying swim-up FLMB fry.

We attribute these high levels of accuracy and precision partly to the experience of the Jensorter operators who had used this electronic device intermittently for at least two years before this study. Because the Jensorter counter also counts air bubbles and debris (Kindschi and Barrows 1991) and its performance is impaired by turbidity or color (Lemaire et al. 1997), clean water and proper operation of the Jensorter to avoid air bubbles are essential to accurate enumeration of fry. Unlike Lemaire et al. (1997), our water was clean and clear, and air bubbles were minimal, if any. Another factor that may have contributed to the accurate enumeration of FLMB fry in this study and walleye fry by Kindschi and Barrows (1991) was anesthetization of the fry. Anesthetized fry moved passively with water flow through the exit ports of the Jensorter, eliminating the inherent tendency of fish to swim against the water current that invariably results in more time and water requirements to get all fry to exit the Jensorter hopper. Lemaire et al. (1997) cited site- and species-specific factors as responsible for the poor accuracy and precision results achieved with striped bass fry. We agree that the use of un-anesthetized fry may have contributed significantly to those poor results by requiring excessive water level manipulation to get all fry to pass through exit holes to be counted.

Verification of the 275 fry/g Standard Gravimetric Index.—The weights of the 73 fry samples used to determine the gravimetric indices were 8–14 g. The mean index for all samples of the entire study period was 289 fry/g and significantly different from the standard index (Table 1). The mean indices for all samples for the early- and late-spring cycles were 284 and 300 fry/g, respectively; both were also statistically higher than the standard index. For age-group 7- 9 d, the index for the early-spring cycle (274 fry/g) and that for the entire study period (277 fry/g) were statistically similar to the standard index whereas that for the late-spring cycle (287 fry/g) was significantly different from the standard index. The younger (<7 d) fry, which were available only in late spring, had a significantly higher index of 315 fry/g. Similarly, the older (≥10 d) fry, available only in early spring, had a significantly higher index of 310 fry/g. The significantly higher indices differed from the standard index by 3.3-14.5%.

The gravimetric index and total length results suggest that all the fry produced in late spring were lighter in weight and shorter compared to the 7-9-d old fry produced in early spring. The 10-d and older fry produced in early spring were as long as the 7-9-d old fish produced in early spring but lighter. Among the 7-9-d fry, those produced in late spring were relatively lighter and shorter than those produced in early spring. Because these fry were produced from two year-classes of broodfish at different water temperatures, parental and temperature effects are likely factors contributing to these observed size differences. However, the two year-classes of broodfish used in this study were propagated from the same genetic line and were in good condition for spawning; thus, we believe parental effect on the results, if any, was inconsequential. Conversely, the temperature difference between early and late spring may explain the results, except that of the 10-d and older fry. The higher temperature of late spring may have increased metabolic rate, requiring more resources to be partitioned to metabolism than to growth of the fry (Colt and Tomasso 2001). For the 10-d and older fry, the lighter weight was probably due to starvation following yolk-sac absorption (Carr 1942; Snow 1975).

The standard index was found to be good for enumerating 7-9-d old FLMB produced either in early spring or over the entire study period, which was the typical fry production period at the A. E. Wood Fish Hatchery. However, the index was inappropriate for estimating numbers of 7-9-d old fish produced in late spring, or numbers of any of the other groups of fry investigated in this study. For example, it was inappropriate for enumerating all fry produced in defined production periods (e.g., early or late spring) if fry size was not taken into consideration. Using the standard index to enumerate all batches or groups of FLMB fry in this study would have resulted in up to 14.5% underestimation of numbers. Consequently, fish inventory numbers would have been inflated and production units (e.g., ponds) overstocked. Overstocking FLMB fry for the production of 51-mm fingerlings can result in early depletion of zooplankton, the primary prey of FLMB fry (Parmley et al. 1986; Tidwell et al. 2000), and adversely affect fish growth and condition. Further, overcrowding of fish in production units can result in disease outbreaks, increased fish mortality, and poor fingerling yield or production. Also, fry distribution numbers would be inflated. For commercial producers, fry sold by the numbers would have been underpriced to result in a financial shortfall. In research aimed at refining fish culture practices, misestimating fry populations would result in data interpretations and conclusions that would be false because the underlining data were inaccurate. Because of these issues, indiscriminate use of standard indices for FLMB fry enumeration should be avoided.

Currently, TPWD staffs enumerate all FLMB fry produced in a season (March-June) when they are 7-10-d posthatch using the standard index of 275 fry/g (Glenewinkel et al. 2011). The results of the present study suggest that the current practice results in underestimation of fry numbers. To improve fry enumeration results, we recommend that the fry must be enumerated at 7-9-d posthatch if the standard index is used. To consistently achieve this size range, eggs incubated together in the same trough should not be more than 2 d postspawn apart.

REFERENCES

Boyd, C. E., and F. Lichtkoppler. 1979. Water quality management in pond fish culture. Research and Development Series 22, Agricultural Extension Service, Auburn University, Alabama.

Carr, M. H. 1942. The breeding habits, embryology and larval development of the largemouth bass in Florida. Proceedings of the New England Zoological Club 20:43-77.

Colt, J. E., and J. R. Tomasso. 2001. Hatchery water supply and treatment. Pages 91-186 *in* G. A. Wedemeyer, editor. Fish hatchery management, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Coutant, C. C., and D. L. DeAngelis. 1983. Comparative temperature-dependent growth rates of largemouth and smallmouth bass. Transactions of the American Fisheries Society 112:416-423.

Davis, J. T., and J. T. Lock. 1997. Largemouth bass: biology and life history. Southern Regional Aquaculture Center Publication No. 200.

Dudley, R. G., and A. W. Eipper. 1975. Survival of largemouth bass embryos at low dissolved oxygen concentrations. Transactions of the American Fisheries Society 104:122-128.

Dupree, H. K., and J. V. Huner. 1984. Propagation of black bass, sunfishes, tilapias, eels, and hobby fishes. Pages 119-135 *in* H. K. Dupree and J. V. Huner, editors. Third report to the fish farmers. U. S. Fish and Wildlife Service, Washington, D. C.

Fry, F. E. J. 1971. The effects of physiological factors on the physiology of fishes. Pages 1-98 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology, volume VI. Academic Press, New York.

Glenewinkel, H., A. Barkoh, T. Engeling, L. Hall, J. Paret, and T. Owens. 2011. Guidelines for the culture of black bass. Texas Parks and Wildlife Department, Management Data Series 267, Austin.

Harrell, R. M., J. H. Kerby, and R. V. Minton. 1990. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Division American Fisheries Society, Bethesda, Maryland.

Hutson, P. L. 1990. Florida largemouth bass culture in Texas. Texas Parks and Wildlife Department, Management Data Series 35, Austin.

Kindschi, G. A., and F. T. Barrows. 1991. Evaluation of an electronic counter for walleye fry. Progressive fish-Culturist 53:180-183.

Lemarie, D. P., D. A. Weller., D. D. Theisen, and L.C. Woods III. 1997. Evaluation of an electronic fry counter with striped bass embryos and larvae. Progressive Fish-Culturist 59:71-74.

Mayes, K. B., P. M. Rosenblum, and T. M. Brandt. 1993. Raceway spawning of Florida largemouth bass: effects of acclimation time and hormone treatment on spawning success. Progressive Fish-Culturist 55:1-8

Moss, D. D., and D. C. Scott. 1961. Dissolved oxygen requirements of three species of fish. Transactions of the American Fisheries Society 90:377-393

Parmley, D., G. Alvarado, and M. Cortez. 1986. Food habits of small hatchery-reared Florida largemouth bass. Progressive Fish-Culturist 48:264-267.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U. S. Fish and Wildlife Service, Washington, D. C.

Snow, J. R. 1975. Hatchery propagation of the black basses. Pages 344-356 *in* H. Clepper and R. H. Stroud, editors. Black bass biology and management. Sport Fishing Institute, Washington, D. C.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. 2nd edition. McGraw-Hill, Inc, New York.

Tidwell, J. H., S. D. Coyle, and T. A. Woods. 2000. Species profile: largemouth bass. Southern Regional Aquaculture Center Publication No. 722.

Table 1.—Mean values of gravimetric index and total length of groups of Florida largemouth bass fry incubated at two water temperature regimes at the A. E. Wood Fish Hatchery, San Marcos, Texas. Values following ± are standard deviations; *p*-values are associated with gravimetric indices.

Age group	*N*	Total length (mm)	Gravimetric index (fry/g)	*P-value*
Early spring (March - April; 19.0-24.7°C)				
< 7-d old	0			
7-9-d old	39	7.27 ± 0.20	274	0.935
≥ 10-d old	12	7.30 ± 0.10	310	0.001
All fry	51		284	0.014
Late spring (May - June; 23.0-27.6°C)				
< 7-d old	10	6.90 ± 0.25	315	0.005
7-9-d old	12	6.90 ± 0.20	287	0.010
≥ 10-d old	0			
All fry	22		300	0.001
Entire study period (March - June; 19.0-27.6°C)				
7-9-d old	51		277	0.538
All fry	73		289	<0.001

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