Factors Determining Quality of Oxytetracycline Marks in Fingerling Walleye Otoliths

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Abstract: This study examined factors which can affect the quality of fluorescent oxytetracycline (OTC) marks on walleye (*Stizostedion vitreum*) otoliths. A 1-time exposure to a strong ultraviolet (UV) light source, such as is done when viewing under a UV microscope, significantly decreased mean OTC mark qualities from an initial value of 2.8 (3.0 is maximum and 0.0 is no mark) to <1.0 3 months following the initial examination. Mark intensity continued to decrease over time (<0.5 after 6 months, 0.3 after 12 months). Otoliths that were stored in an unlit environment consistently had higher intensity marks over time than those stored in a lighted environment. Dimethyl sulfoxide (DMSO) was examined as a possible potentiator to improve OTC mark intensity. Otoliths that were marked by immersion in 200 ppm OTC and 0.81% DMSO did not exhibit higher quality marks than those which were immersed only in 200 ppm OTC. Fish growth rate immediately surrounding the time of marking appeared to affect the quality of OTC marks. In addition, Walleyes that were fed to satiation throughout the study possessed a mean mark quality of 2.6, while those that were starved or fed 1% of their body weight/day had mark qualities of 2.0 or 1.9.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 50:174-181

Determining factors that affect marking procedures and mark recognition are important if a mark will be used to assess relative contribution of stocked fish to a population. Mark degradation, fish growth rate, and the use of a potentiator are some important parameters that could affect the quality of an oxytetracycline (OTC) mark. Exposing marked otoliths to fluorescent light may cause degradation of the fluorescent rings produced from the marking procedure. Since OTC binds to calcium structures during ossification, fast-growing fish may incorporate more OTC into their otoliths and exhibit brighter marks than slow-growing fish. The use of a potentiator during marking may increase the intensity of the marks by augmenting the uptake of OTC.

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Few papers have dealt with the effects of immersion marking procedures of walleye and the resultant quality of marks. Schademann (1987), in one effort to assess the reliability of the technique, immersed walleye fry (80 hours after swim up) in 200 ppm tetracycline (TC) and 1% dimethyl sulfoxide (DMSO) for 24 hours. Fifty percent of the fish had OTC marks in the caudal bones, but the study did not provide for the examination of otoliths. In the same study, 70% of the fish immersed for 24 hours 104 hours after swim up with 200 ppm TC and 1.5% DMSO had concentric fluorescent rings near the middle of their caudal bones. There were no marks on the cleithra. Mortality increased when >2.5% DMSO was used. Structures began to show fluorescence when immersion was initiated approximately 75 hours after swim up.

Scidmore and Olson (1969) immersed 50- to 75-mm juvenile walleyes in various concentrations of OTC and DMSO. Marks were not produced at concentrations <200 ppm OTC for 8-hour immersions. They did not examine otoliths. Water temperature and hardness had little effect on mark intensity. Pelvic bones showed more of a consistent mark than ribs, centra, or neural spines. Boiling the fish for 2–3 minutes to remove bones had little effect on the mark. They recommended large-scale marking be conducted at temperatures <15.5 C to avoid high mortality. Kayle (1992) found immersing fingerling walleyes in 200 ppm OTC and 0.81% DMSO for 8.5 hours resulted in 100% mark retention for 158 days. The fish were examined with a portable 100-W, UV light and a binocular microscope. Kayle only looked at 56 fish and did not use any unmarked fish as controls. Brooks et al. (1994) worked extensively on marking otoliths of larval and juvenile walleyes by immersion in OTC. They were able to mark 100% of the fish with 500 ppm OTC. They were unable to mark 100% of the fish in 200 ppm OTC. They suspected some relationship exists between the growth rate of the fish, storage of the otolith, and the ease of reading the mark.

The objectives of this study were to determine 1) the effects of a 1-time exposure to UV light on mark quality, 2) the effects of particular storage methods on mark quality, 3) growth rate before an after marking, and 4) the effectiveness of DMSO as a potentiator in improving OTC mark quality in walleye.

This research was funded by the Southern Illinois University, Cooperative Fisheries Research Laboratory. Fingerling walleyes were obtained in Illinois from the Commonwealth Edison Company (LaSalle Hatchery) and from the Lake Rathbun Fish Hatchery of the Iowa Department of Natural Resources. Thanks are extended to Ronald Brooks for his advise on chemical immersion marking and data analyses, and Bradd Sims and Eric Sandberge for their time and effort in harvesting fingerling walleyes from culture ponds.

Methods

OTC Mark Degradation and Storage Methods

Fingerling (50mm total length) Walleyes were marked with 500 mg/liter buffered OTC for 6 hours as described by Brooks et al. (1994). The marked fingerlings were stocked in June 1994 into 3 0.07-ha research ponds (N = 150/pond) at Southern

Illinois University, Carbondale, Touch of Nature Fisheries facility. At the same time 3 0.07 ha ponds were stocked with unmarked fingerlings at the facility to serve as controls. A sample of 270 walleyes was taken from each of the groups (marked and control) after 4 months. The sagittal otoliths of 30 walleyes from each group were removed and 1 of the 2 otoliths was examined immediately with a UV microscope, thus exposing the examined otoliths to strong, 100-W UV light for a total of about 1 minute. Both otoliths were then stored in dry vials in the dark and reexamined at either 3, 6, or 12 months later. Otoliths were randomly assigned to these time intervals. The otoliths from an additional 30 marked walleyes and 30 unmarked walleyes were extracted and placed in dry vials for frozen storage. They were then examined under a 100-W UV light for a total of approximately 1 minute. After initial examination, otoliths were immediately returned to a freezer and randomly assigned to the 3 time intervals for subsequent examinations at either 3, 6, or 12 months. The otoliths from the other 420 walleyes (210 marked and 210 controls) were subjected to 7 storage methods for observation after 3, 6, or 12 months. In the first storage method, the otoliths were left in 60 fish (N = 10 marked and 10 controls/time interval) and the fish were kept frozen until examination, at which time the otoliths were removed and examined under UV light immediately. In the other 6 storage methods (N = 30 marked and 30 controls), otoliths were removed when fish were harvested from the ponds and placed either in a clear dry vial in the light, a clear dry vial in darkness, a vial filled with glycerin in a lighted environment, a vial filled with glycerin in a dark environment, a scale envelope stored in the dark, or a scale envelope stored in lighted conditions. Twenty different otoliths (10 marked and 10 controls) from each storage method were examined at 3, 6, or 12 months. Otoliths stored in the light were exposed to standard laboratory fluorescent lighting for approximately 8 hours/day. The laboratory had no windows and was lighted by 8 60-W fluorescent bulbs.

Otoliths were examined using a Nikon Optiphot 2 compound microscope equipped with a 100-W UV light source, 450- to 490-nm exciter filter, 410-nm barrier filter, and a 415 dichroic mirror. This was the same system used by Bumguardner (1991) to examine red drum (*Sciaenops ocellatus*) otoliths and by Brooks et al. (1994) to examine walleye otoliths for the presence of OTC marks. Otoliths were mounted on glass microscope slides using cyanoacrylic glue. All otoliths were examined in a random blind manner.

OTC mark intensity on otoliths was ranked subjectively as no visible mark, poor, fair, or good with corresponding numerical values of 0, 1, 2, or 3, respectively. All otoliths were examined and ranked by 1 individual to maintain consistency. OTC mark quality among all groups was compared with an analysis of variance procedure followed by Duncan's Multiple Range Test to determine an optimal storage method. All statistical tests used a significance level of 0.05.

DMSO as a Potentiator in Improving Mark Quality

Fingerling walleyes (50 mm) were marked with buffered OTC using 3 different protocols and stocked into 3 different 0.07-ha research ponds (N = 200/pond) in June 1994 at Southern Illinois University, Carbondale, Touch of Nature Fisheries facility.

A fourth pond was stocked with unmarked walleyes as controls. Walleyes were marked as described by Kayle (1992), by immersion in 200 mg buffered OTC/liter without DMSO for 8 hours, and by immersion in 500 mg buffered OTC/liter without DMSO for 6 hours. Twenty walleyes were sampled from each pond every month for 6 months. When walleyes were harvested from the ponds, the otoliths of marked and unmarked fish were removed and immediately examined under a UV compound microscope using the same procedure described earlier. Comparisons of mark quality between the 4 groups were made as described above to determine the effectiveness of DMSO as a potentiator.

Growth Rate and OTC Mark Quality

Advanced fingerling walleyes trained on prepared feed were obtained from the Lake Rathbun Fish Hatchery, Iowa. Walleyes (N = 120) of approximately the same total length (155 mm) and weight (29 g) were selected for this experiment. The fish were held in circular tanks (diameter = 1.85 m) under ambient light conditions. Once they were acclimated to 20 C, experimental feeding rates were initiated. There were 4 feeding treatments (N = 30/treatment) and all fish were held at 20 C. Walleyes were fed varying rations of pelleted feed (Silvercup, 37% crude protein) during the experiment. Fish in Treatment 1 were deprived of food for 5 days prior to marking. After marking the fish were fed to satiation twice a day for the remainder of the 60-day experiment. Treatment 2 fish were maintained on a minimum ration (1% body weight/day) of feed for 5 days before marking and 5 days after marking, then fed to satiation twice daily until termination of the study. Treatment 3 fish were fed to satiation twice a day for 5 days prior to marking, then deprived of food for 5 days following marking. Upon completion of the deprivation period the fish were fed to satiation as in Treatments 1 and 2. Treatment 4 fish were fed twice daily to satiation for the duration of the experiment. Walleye fingerlings were marked by immersing for 6 hours in 500 ppm buffered OTC. All fish were held in the circular tanks for 30 days following marking and then the sagittae were removed and examined immediately for OTC mark intensity. The quality of the fluorescent OTC mark was subjectively ranked as previously described. Mean lengths and weights for each treatment group were calculated at the beginning and end of the experiment.

Growth rates and mark quality were analyzed as described above.

Results and Discussion

Degradation and Storage

Ultraviolet Light Exposure. Initially 100% of the walleyes immersed in 500 mg/liter OTC were marked. These otoliths that were examined under a strong ultraviolet light and subsequently placed in frozen storage or dark storage possessed mean mark qualities of 2.7 and 2.8, respectively (Table 1). Ensuing examinations at 3-, 6-, and 12-month intervals revealed a significant decline in mark quality from the initial examination to the 3-month interval for otoliths stored in a dark environment and for

Table 1. Effects of a 1-time exposure to a strong UV light source on mean OTC mark quality rated from 0 (nonvisible mark), 1 (poor), 2 (fair), to 3 (good visibility) for walleye otoliths over time (months). The initial exposure, for the 2 exposed groups, was at 0 months. SD in parentheses.

	Mean mark quality[a]			
Storage method	0	3	6	12
Unexposed dark	2.8 (0.31)A	2.6 (0.32)A	2.5 (0.29)A	2.5 (0.33)A
Unexposed frozen	2.7 (0.32)A	2.5 (0.28)A	2.5 (0.30)A	2.5 (0.31)A
Exposed dark	2.8 (0.29)A	0.8 (0.65)B	0.4 (0.71)B	0.3 (0.63)B
Exposed frozen	2.8 (0.32)A	0.9 (0.48)B	0.3 (0.64)C	0.3 (0.59)B,C
Controls	0.0 (0.0)D	0.0 (0.0)D	0.0 (0.0)D	0.0 (0.0)D

[a]Column and row means with different letters are significantly different ($P < 0.05$).

those placed in frozen storage. Mark quality of otoliths not exposed to UV prior to storage did not differ after 3, 6, and 12 months in storage. There was also an additional significant decline in mark quality over the time period of 3–6 months for those otoliths that were placed in frozen storage. The decline in mark quality of otoliths placed in dark storage from 3–6 months was not significant. There were only slight declines in mean mark quality from 6 months to 12 months for both storage methods.

These results indicate a 1-time exposure to a strong UV light source causes significant deterioration of OTC marks on fingerling walleye otoliths. Furthermore, the majority of this degradation appears to occur within 3 months following exposure to ultraviolet light.

Storage Methods. Otoliths stored in a dark environment had a significantly higher mark quality than those exposed to standard fluorescent lighting after 3, 6, and 12 months (Table 2).

There were no significant decreases in mean mark quality among the storage times for otoliths stored in dark or lighted environments. Mark quality in otoliths

Table 2. Effects of different storage methods on the quality of OTC marks rated from 0 (no visible mark), 1 (poor), 2 (fair) to 3 (good visibility) over time (months). SD in parentheses.

	Mean mark quality[a]		
Storage method	3	6	12
In fish	2.8 (0.31)A	2.8 (0.32)A	2.7 (0.33)A
Envelope in dark	2.8 (0.34)A	2.7 (0.36)A	2.6 (0.38)A
Glycerin vial in dark	2.8 (0.34)A	2.5 (0.37)A	2.8 (0.32)A
Dry vial in dark	2.7 (0.35)A	2.7 (0.36)A	2.5 (0.38)A
Envelope in light	1.6 (0.69)B	1.4 (0.75)B	0.8 (0.88)B
Glycerin vial in light	1.3 (0.71)B	0.7 (0.95)C	0.4 (0.89)B
Dry vial in light	1.1 (0.73)B	0.8 (0.76)C	0.4 (0.80)B
Controls	0.0 (0.00)D	0.1 (0.35)D	0.0 (0.00)D

[a]Column and row means with different letters are significantly different ($P < 0.05$).

stored in the light decreased with time more than did mark quality in otoliths stored in the dark (Table 2). Therefore, long-term exposure to a low-intensity light source will induce degeneration of OTC marks.

DMSO as a Potentiator in Improving OTC Mark Quality

There are contradictory reports on the effectiveness of using DMSO as a potentiator for OTC. Kayle (1992) reported 100% marks on otoliths of walleyes immersed in 200 mg OTC/liter and 0.81% DMSO for 8 hours. He also reported mark retention after 158 days. Brooks et al. (1994) duplicated Kayle's protocol twice and found that <3% of the otoliths examined exhibited a mark after 30–38 days. We found that otoliths marked using 500 ppm OTC without DMSO displayed high quality marks ($\bar{x} = 2.5$) over the 180-day study, while those marked following the other 2 procedures had significantly lower mark qualities ($\bar{x} = 0.1$ when immersed in 200 ppm OTC and 0.81% DMSO, and $\bar{x} = 0.09$ when immersed in 200 ppm OTC alone) (Table 3). Differences in mark quality between the 2 groups marked with 200 ppm OTC were not significant at any of the 30-day intervals or after 180 days.

Only 7.5% of the walleyes marked using 200 ppm OTC with 0.81% DMSO (Kayle 1992) possessed otoliths that exhibited marks. These results are similar to those reported by Brooks et al. (1994) using Kayle's marking procedure. In this experiment, approximately 6.7% of the walleyes immersed in 200 ppm OTC without DMSO were marked. Marks observed from these 2 protocols were usually of low intensity with 9 ranked as fair, 16 ranked as poor, and none ranked as good. In contrast, 100% of otoliths from fish immersed in 500 ppm OTC possessed marks. The quality of these marks remained high over the 6-month period with means ranging from 2.6 (month 1) to 2.5 (months 4, 5, and 6).

These results demonstrate DMSO was not an effective potentiator in improving OTC mark quality and only a very low percentage of fish immersed in 200 ppm OTC with or without DMSO would be marked.

Effect of Growth Rate on OTC Mark Quality

Fish fed to satiation throughout the experiment had significantly larger increases in both total length and weight gain than the other 3 groups (Table 4). The faster

Table 3. Effectiveness of DMSO as a potentiator in improving OTC mean mark quality rated from 0 (no visible mark), 1 (poor), 2 (fair) to 3 (good visibility). Otoliths were extracted and examined immediately. SD in parentheses.

	Mean mark quality[a]					
Treatment	1	2	3	4	5	6
500 ppm OTC	2.65 (0.32)A	2.55 (0.33)A	2.60 (0.35)A	2.50 (0.37)A	2.50 (0.38)A	2.50 (0.34)A
200 ppm OTC	0.25 (0.43)B	0.18 (0.27)B	0.05 (0.20)B	0.01 (0.15)B	0.05 (0.18)B	0.00 (0.00)B
200 ppm OTC and DMSO	0.35 (0.57)B	0.10 (0.22)B	0.05 (0.18)B	0.05 (0.20)B	0.00 (0.00)B	0.05 (0.18)B

[a]Column means with different letters are significantly different ($P < 0.05$).

Table 4. Increases in total length and weight and mean mark quality rated from 0 (no visible mark), 1 (poor), 2 (fair) to 3 (good visibility) of fingerling walleyes marked with OTC after being subjected to different feeding protocols. Following time periods when feeding rates were manipulated, fish were fed to satiation. SD in parentheses. Column means with different letters are significantly different ($P < 0.05$).

Treatment before marking	*N*	Mean increase in TL (mm)	Mean increase in weight (g)	Mean mark quality
Fed to satiation	30	13.10 (7.1)A	10.64 (6.8)A	2.77 (0.31)A
Starved 5 days before	30	6.23 (4.3)B	4.92 (3.2)B	2.17 (0.38)B
Starved 5 days after	29	5.07 (4.4)B	4.58 (3.8)B	2.10 (0.41)B
Fed 1% body weight/day	28	4.93 (3.8)B	4.57 (4.0)B	2.14 (0.43)B

growing fish had a mean mark quality of 2.6 which was significantly higher than the mean mark qualities of either 2.0 or 1.9 exhibited by the other 3 groups.

These results indicate the quality of OTC marks appears to be related to the growth rate of the fish before and after marking. However, growth rates had a much smaller effect than exposure to light.

Summary and Conclusions

Exposure of OTC-marked otoliths to a strong UV light source precipitated a fairly rapid degeneration in mark quality. Mean mark qualities significantly declined in the first 3 months of storage after exposure to a 100-W UV light source for otoliths stored in both dark and frozen conditions. This decline continued from 3 to 6 months and from 6 to 12 months, but at a slower rate. Otoliths that were not initially exposed to a strong UV light source maintained high quality marks over the 12-month period of the study. Therefore, if OTC-marked otoliths are going to be placed into storage for subsequent examination, exposure to ultraviolet light should be avoided until the time of inspection for the OTC mark.

The type of storage method that marked otoliths are placed into appears to have an effect on the quality of OTC marks. Otoliths that were placed in storage that protected them from long-term exposure to low-intensity fluorescent light exhibited significantly higher mean mark rankings than those that were not protected from these lighting conditions over the entire 12-month study period. Consequently, it is important that otoliths which are not going to be immediately examined be protected from long-term exposure to low-intensity fluorescent lighting. Although the effects of exposure to sunlight were not tested, it is highly likely that it would also cause degradation of OTC marks.

The percentage of otoliths marked and the quality of these marks were similar for walleyes immersed in 200 ppm OTC with and without DMSO. Fingerling walleyes marked in 500 ppm OTC without DMSO (Brooks et al. 1994) resulted in 100% of the otoliths possesesing high quality marks. Therefore, use of DMSO as a potentiator is not recommended.

Growth rates immediately before and after the time of marking had a significant effect on the quality of OTC marks. Fish that were fed to satiation throughout the entire study displayed higher rates of growth and a higher mean mark quality than the other 3 treatment groups. These results indicate that slower growing fish can be marked using OTC, but the resultant marks are likely to be of lower quality and more difficult to detect than would be normally expected.

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