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Eric G. Unkenholz
South Dakota State University

Michael L. Brown
South Dakota State University

Kevin L. Pope
South Dakota State University, kpope2@unl.edu

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Oxytetracycline Marking Efficacy for Yellow Perch Fingerlings and Temporal Assays of Tissue Residues

ERIC G. UNKENHOLZ, MICHAEL L. BROWN,[1] AND KEVIN L. POPE

Department of Wildlife and Fisheries Sciences, South Dakota State University Brookings, South Dakota 57007-1696, USA

Abstract.—Differentiating between hatchery and naturally reproduced fishes is difficult because of the lack of appropriate marking techniques. Chemical immersion techniques can be a practical method for mass marking juvenile fishes. The objectives of this study were to determine the concentration of oxytetracycline (OTC) hydrochloride needed to effectively mark age-0 yellow perch *Perca flavescens*, to observe the retention time of the mark, and to measure the persistence of OTC in body tissues. Fish were immersed in 309, 534, and 748 mg OTC/L for 12 h. Initial fish collections were made weekly, and monthly collections began 1 month postmarking for tissue and mark analyses. High-pressure liquid chromatography was used to quantitate OTC residues in tissues; OTC concentrations were undetectable (<0.05 μg/g) in the edible tissue (muscle) at 110 d postimmersion. Aided by a Nikon Labophot fluorescence microscope, we discerned otolith marks 56 d postimmersion on 100% of the fish treated at 534 and 748 mg/L. Marks were still visible on 100% of the fish treated with 534 and 748 mg/L at 8 months postimmersion.

Fish fry and fingerling stocking programs are important components of many natural resource agency operations (Schramm and Piper 1995). However, obtaining evidence on the success of stocking efforts, other than introductory stockings, is usually difficult because of problems differentiating hatchery-produced fish from naturally produced fish. Typically, conventional marking and tagging techniques cannot be used because of the small size and large number of fish to be stocked (Nielsen 1992).

Chemical and genetic markers are essentially the only tools currently available to fisheries biologists for the evaluation of large-scale stockings of small fish (Nielsen 1992). Although the use of genetic markers for large-batch marking is possible, the process is costly and time consuming because it involves highly technical selection, isolation, and manipulation of allele frequencies (Nielsen 1992). Currently, chemical batch marking appears to be the most viable approach for evaluation of stocking.

Several chemicals have been evaluated for marking various fish species at different life stages (e.g., Hettler 1984; Younk and Cook 1991; Nielsen 1992; Brooks et al. 1994) with the objective of implementing these marking techniques in stocking programs (Weber and Ridgway 1967; Lorson and Mudrak 1987; Kayle 1992). Elements or compounds evaluated have included alkali metals (cesium, rubidium), alkaline earths (strontium, barium), transition metals (manganese), rare earths (samarium, europium, terbium), antibiotics (tetracyclines), and calcein (Behrens Yamada and Mulligan 1990). The utility of various chemical marking approaches varies considerably; however, fluorescent chemicals, such as tetracycline (TC) and oxytetracycline (OTC), appear to perform best. They provide convenient modes of induction, relatively long-term marks, and low-cost detection when compared with other available approaches (Younk and Cook 1991; Nielsen 1992).

Several studies have involved the mass marking of juvenile fishes with tetracycline compounds (e.g., Younk and Cook 1991). The most common tetracycline-based formulations reported in the literature are TC, TC hydrochloride, and OTC hydrochloride. Successful results have been achieved by administering tetracyclines by direct injection, by feeding diets containing the chemical, or by immersing fish in a solution. The most appropriate induction method is dictated by fish size and life stage, the quantity of fish to be marked, facilities and equipment required, budget and time limitations, and any other factors unique to a particular study. However, immersion is the most common method because it is easily applied to any life stage (Younk and Cook 1991).

Larval through juvenile stages of various fishes have been successfully marked by immersion in OTC or TC concentrations ranging from 100 to 500 mg/L for durations of 3–24 h (Weber and Ridgway 1962; Choate 1964; Scidmore and Olson 1969; Lorson and Mudrak 1987; Muth and Bestgen 1991; Secor et al. 1991; Brooks et al. 1994). De-

[1] Corresponding author: brownm@mg.sdstate.edu

tection of fluorescent marks is dependent upon structures examined, age of fish at immersion, experience of the examiner, and the sensitivity of the viewing equipment (Campana and Neilson 1982; Hettler 1984; Dabrowski and Tsukamoto 1986). For example, Brooks et al. (1994) reported fluorescent marks on 100% of the otoliths extracted from walleyes *Stizostedion vitreum* (fry and fingerlings) immersed at 350–500 mg OTC/L for 6 h. Fielder (1994) reported a 63% marking success on fingerling walleyes, using similar methods but different filters in the viewing equipment. Secor et al. (1991) detected fluorescent marks on 100% of the otoliths in OTC-treated larval striped bass *Morone saxatilis*, but they reported inconsistent results for juveniles.

We investigated OTC-immersion marking of yellow perch *Perca flavescens* because a method was needed to document the effectiveness of yellow perch stockings. Also, OTC is regulated as an investigational new animal drug by the U.S. Food and Drug Administration, and more data are needed about residual OTC in fish tissues. Specifically, the objectives of this study were to determine the concentration of OTC needed to effectively mark age-0 yellow perch, the retention time in sagittal otoliths, and the persistence of OTC in body tissues.

Methods

Yellow perch fingerlings (90–120 d old, 35–60 mm total length, TL) were used in this study. A control group was kept for a survival reference and to determine observer detection bias. The remaining fish were randomly divided into four groups and placed in separate 1,250-L fiberglass raceways (N = 516 fish/raceway). Solutions of OTC hydrochloride ($C_{22}H_{24}N_2O_9{\cdot}HCL$), buffered to the control pH (7.2) with sodium phosphate (dibasic, Na_2HPO_4), were added to each raceway to give calculated final concentrations of 300, 500, or 700 mg OTC/L (the control raceway received no OTC). An antifoam agent (5% silicone base, food grade) was added (60 mL/raceway) to minimize foaming of the OTC solution. Treatment continued for 12 h, and each treatment group was subsampled (N = 10–12 fish) at 3, 6, 9, and 12 h to determine OTC uptake relative to immersion time. After the immersion-marking period, normal water flows were resumed and the OTC-treated waters were flushed through an active carbon filter to remove the organic contaminant.

Our water source for marking and holding was a municipal supply that was treated with sodium thiosulfate to remove chlorine. Total hardness was 381.1 (±37.9 SD) mg/L as $CaCO_3$. The marking and holding temperature was 12°C through 217 d. After 217 d, water temperature was maintained at 22°C. During the holding period, water quality was maintained with a flow rate of 3.75 L/min and supplemental aeration. Fish were fed a prepared grower diet (BioDiet, Warrenton, Oregon) three times daily from an electronic feeder. Average weight of three subsamples per raceway was determined biweekly, and feed rations were adjusted to 7% of body weight. A photoperiod of 13 h light : 11 h dark was maintained throughout the study.

Initial fish collections were taken weekly, and monthly collections began 1 month postmarking for tissue and mark analyses. Ten fish were collected from each treatment on each sample date. The samples were stored in the dark and frozen at −20°C pending preparation and analysis. Individual fish from each treatment were prepared by removing the head, viscera, and fins. The remaining tissue was pooled and homogenized to form a composite sample for each treatment and date (7, 14, and 42 d). Thus, each composite sample contained both edible (muscle) and inedible (skin, scales and bones) tissues. At 110 d, we analyzed composite samples and separate samples of edible tissues.

High-pressure liquid chromatography (HPLC) was used to determine OTC residues in tissues according to the methods of Iwaki et al. (1992). The limit of quantitation for OTC in fish is 0.05 μg/g by this method. Control fish samples were determined to contain 0.00 μg OTC/g. An untreated fish sample was spiked with 0.17 μg OTC/g and determined to contain that concentration. Tissue concentration data were tested with analysis of variance; means were compared with the Tukey honestly significant difference procedure.

Sagittal otoliths were removed and affixed, sulcus side down, to slides with cyanoacrylic cement. Wetted sandpaper (600 grit) was used to prepare otoliths for mark detection. Autofluorescence was detected according to the methods of Brooks et al. (1994) and Fielder (1994) with the aid of a Nikon Labophot fluorescence microscope equipped with a 100-W ultraviolet light source, 450–490 nm excitation filter, a BA-515IF barrier filter, and a 510-nm dichroic mirror. Two examiners independently viewed the otoliths for mark presence or absence.

Results and Discussion

Analysis of the treatment solutions at 6 h showed concentrations of 309, 534, and 748 mg OTC/L for 300, 500, and 700 mg/L treatments,

TABLE 1.—High-pressure liquid chromatography assays of oxytetracycline residues (μg OTC/g tissue) in yellow perch fingerlings following immersion in 309, 534, or 748 mg OTC/L. Analyses were from replicates of composite samples (inedible and edible tissues, $N = 10$) of yellow perch fingerlings immersed for 3, 6, 9, and 12 h. Column means without a letter in common are significantly different ($P < 0.05$).

Immersion concentration (mg OTC/L)	Immersion time (h)	Mean assayed concentration (μg OTC/g tissue)	SD
309	3	1.82 yx	0.51
	6	3.56 zy	0.13
	9	3.40 zyx	0.71
	12	2.20 zyx	0.16
534	3	2.50 zyx	0.77
	6	3.12 zyx	0.54
	9	2.53 zyx	0.57
	12	3.16 zyx	0.23
748	3	1.55 x	0.03
	6	3.34 zyx	0.06
	9	1.96 yx	0.19
	12	4.10 z	0.37

TABLE 2.—Temporal high-pressure liquid ($N = 10$) chromatography assays of oxytetracycline (μg OTC/g tissue) in composite samples ($N = 10$) of yellow perch tissues following immersion treatment in OTC.

Immersion concentration (mg OTC/L)	Days after marking		Assayed concentration (μg OTC/g tissue)
	Composite[a]	Edible[b]	
309	7		0.59
	14		0.66
	42		0.42
	110		0.57
		110	<0.05
534	7		0.82
	14		1.06
	42		0.41
	110		0.56
		110	<0.05
748	7		0.63
	14		0.89
	42		0.43
	110		0.44
		110	<0.05

[a] Nonedible and edible tissue (i.e., skin, scales, bones, muscle) combined.
[b] Muscle tissue only.

respectively; therefore, calculated OTC concentrations were similar to assayed levels. Residual OTC in fish collected at 3, 6, 9, and 12 h showed a peak at 6 h (Table 1), with all treatment assays exceeding 3 μg/g tissue. After 6 h, all concentrations remained constant or decreased, except for the 748 mg/L treatment at 12 h.

Mortality was monitored during the marking and rearing period. No mortality occurred in any of the treatments during the first 72 h. Total mortalities determined at the conclusion of the study were 1.1% for the control and 0.4%, 0.5%, and 0.9% for the treatments at 309, 534, and 748 mg/L, respectively. Brooks et al. (1994) found that water temperature significantly influenced mortality during OTC treatment (mortality was greater at higher temperatures) but that concentration and duration did not. Peterson and Carline (1996) found that tetracycline marking did not affect the short-term survival of walleye fry. Thus, it appears that immersion in OTC has little or no effect on survival as long as the water quality characteristics are kept within normal ranges for the species. In all, 225 fingerlings were harvested during the study for tissue analysis or mark detection. All fish appeared to be healthy at harvest.

One of the problems with the use of OTC as a marking agent for sport fishes is the potential OTC residual in fillets (muscle) that may be consumed by anglers. Treated fingerlings were successfully reared and periodically harvested for analysis to determine the persistence of OTC in body tissues through 110 d postimmersion (Table 2). That analysis showed OTC concentrations in edible tissues were undetectable (<0.05 μg/g) compared with samples containing the nonedible portion (Table 2). At 110 d, the treated fish averaged 72 mm TL. Normally, yellow perch are not harvested for human consumption until they exceed 150 mm TL. Although retention of OTC in the nonedible portion could be an important consideration for a species, such as Pacific salmon *Oncorhynchus* spp., that is canned with bones left in the product, fillets are the only consumable portion of most sportfish species.

Otoliths removed at 42 and 56 d postimmersion were used to check for mark detection. Fluorescent marks were produced as a result of immersion in OTC. No marks were found on the control specimens; marks were present in 100% of the otoliths from fish treated with OTC at 534 and 748 mg/L at both 42 and 56 d postmarking in blind comparisons (Table 3). There was complete agreement (100%) between the two examiners on mark presence or absence. An average of 33% detection resulted from the fish treated at 309 mg/L for the same dates. Marks appeared as a yellowish gold band following the daily growth ring contour. Because of slow growth following immersion, the position of the mark was near the margin of the otolith in all cases and was most easily found on the anterior or posterior ends. In most cases, three to six swipes of sandpaper were required to reveal

TABLE 3.—Percent of marks detected in otoliths of fingerling yellow perch (N = 10 per treatment and date) immersed in oxytetracycline (OTC).

Immersion concentration (OTC mg/L)	Number of days after marking	Percent with visible marks
0	42	0
	56	0
	217	0
309	42	40
	56	20
	217	80
534	42	100
	56	100
	217	80
	247	100
748	42	100
	56	100
	217	100
	247	100

the mark. Overall quality of the marks was fair to good, and the time required to locate marks was 1–6 min/otolith. Otoliths collected 217 d postimmersion were also evaluated for mark retention. Marks were observed on 80% of the otoliths for fish treated at 534 mg/L and 100% of the otoliths treated at 748 mg/L.

Although we did achieve 100% mark recognition for 217 d at 748 mg/L, we were concerned about not detecting the complete mark. We attributed this problem to slow growth due to the low water temperatures (average, 12°C) in the raceways through most of this study. From 12 September 1995 to 19 March 1996, the average weight of yellow perch increased 0.6 g/fish. The preferred temperature range for yellow perch is 21–24°C (Scott and Crossman 1973), however optimum growth occurs between 23 and 28°C (Hokanson 1977). Therefore, at day 217 postimmersion we added heaters to the raceways to cause an increase in food uptake, growth rate, and otolith dimensions, which would cause the mark to be located further from the margin of the otolith.

Temperature during the last month of the study averaged 22°C. Average weight of fish increased from 4.16 g to 8.42 g in 30 d. Because of the increase in otolith size, the marks became more visible as a complete ring, but in general, mark intensity remained the same. It appears that the increase in otolith size due to increased water temperature contributed to mark detection but not mark quality. This could account for the increase in mark detection from 80% at 217 d to 100% at 247 d postimmersion for fish treated at 534 mg/L (Table 3). At 217 and 247 d, marks were recognized on 100% of the otoliths extracted from fish treated at 748 mg/L.

In conclusion, immersion in OTC provided a viable technique for mass-marking juvenile yellow perch. Potentially, the use of this technique will allow stocked juvenile yellow perch to be differentiated from naturally reproduced fish. Based on these OTC uptake and mark detection data, we believe that an immersion treatment for a minimum of 6 h in a buffered solution of 500 mg OTC/L will provide a reliable mark for stocking studies lasting a moderate length of time (i.e., at least 8 months).

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References

Behrens Yamada, S., and T. J. Mulligan. 1990. Screening of elements for the chemical marking of hatchery salmon. Pages 550–561 *in* N. C. Parker, and five coeditors. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Brooks, R. C., R. C. Heidinger, and C. C. Kohler. 1994. Mass marking otoliths of larval and juvenile walleyes by immersion in oxytetracycline, calcein, or calcein blue. North American Journal of Fisheries Management 14:143–150.

Campana, S. E., and J. D. Neilson. 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some environmental variables in their production. Canadian Journal of Fisheries and Aquatic Sciences 39:937–942.

Choate, J. 1964. Use of tetracycline drugs to mark advanced fry and fingerling brook trout (*Salvelinus fontinalis*). Transactions of the American Fisheries Society 93:309–311.

Dabrowski, K., and K. Tsukamoto. 1986. Tetracycline marking in coregonid embryos and larvae. Journal of Fish Biology 29:691–698.

Fielder, D. G. 1994. An evaluation of the suitability of marking walleye fry and fingerlings with oxytetracycline. South Dakota Department of Game, Fish and Parks, Fisheries Completion Report 94-15, Pierre.

Hettler, W. F. 1984. Marking otoliths by immersion of

marine fish larvae in tetracycline. Transactions of the American Fisheries Society 113:370–373.

Hokanson, K. E. F. 1997. Temperature requirements of some periods and adaptations of the second cycle. Journal of the Fisheries Research Board of Canada 34:1524–1550.

Iwaki, K., N. Okumura, and M. Yamazaki. 1992. Determination of tetracycline antibiotics by reversed-phase high-performance liquid chromatography with fluorescence detection. Journal of Chromatography 623:153–158.

Kayle, K. A. 1992. Use of oxytetracycline to determine the contribution of stocked fingerling walleyes. North American Journal of Fisheries Management 12:353–355.

Lorson, R. D., and V. A. Mudrak. 1987. Use of tetracycline to mark otoliths of American shad fry. North American Journal of Fisheries Management 7:453–455.

Muth, R. T., and K. R. Bestgen. 1991. Effect of sunlight on tetracycline marks in otoliths of Colorado squawfish larvae. Transactions of the American Fisheries Society 120:666–668.

Nielsen, L. A. 1992. Methods of marking fish and shellfish. American Fisheries Society, Special Publication 23, Bethesda, Maryland.

Peterson, D. L., and R. F. Carline. 1996. Effects of tetracycline marking, transport density, and transport time on short-term survival of walleye fry. Progressive Fish-Culturist 58:29–31.

Schramm, H. L., Jr., and R. G. Piper, editors. 1995. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Scidmore, W. J., and D. E. Olson. 1969. Marking walleye fingerlings with oxytetracycline antibiotics. Progressive Fish-Culturist 31:213–216.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184.

Secor, D. H., M. G. White, and J. M. Dean. 1991. Immersion marking of larval and juvenile hatchery-produced striped bass with oxytetracycline. Transactions of the American Fisheries Society 120:261–266.

Weber, D. D., and G. J. Ridgway. 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. Progressive Fish-Culturist 24:150–155.

Weber, D. D., and G. J. Ridgway. 1967. Marking Pacific salmon with tetracycline antibiotics. Journal of the Fisheries Research Board of Canada 24:849–865.

Younk, J. A., and M. F. Cook. 1991. Fluorescent chemical marking of walleye larvae with a selected literature review of similar investigations. Minnesota Department of Natural Resources, Fisheries Investigational Report 48, St. Paul.

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