The Efficacy of Mass-marking Channel Catfish Fingerlings by Immersion in Oxytetracycline

David R. Stewart*
Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, Oklahoma State University, 07 Ag Hall, Stillwater, OK 74078, USA

James M. Long
U.S. Geological Survey, Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, Oklahoma State University, 007 Ag Hall, Stillwater, OK 74078, USA

Oxytetracycline (OTC) has been extensively used for marking a variety of fish species, but has never been successfully used to mark channel catfish *Ictalurus punctatus*. Channel catfish fingerlings (~ 25 mm TL) obtained from the Oklahoma Department of Wildlife Conservation at Byron Fish Hatchery were kept in Living Streams (791 to 1,018 L) equipped with recirculation units. Marking trials consisted of immersing channel catfish in one of three concentrations (250, 450, and 700 mg/L) OTC hydrochloride [HCl] for 6 hours. Samples of channel catfish were obtained from each group at 1-week and 4-week post-immersion. Lapilli otoliths and pectoral spines were removed to assess mark presence with an epi-fluorescent compound microscope. After one week, no marks were detected on pectoral spines for all treatments, mark detection on otoliths depended on concentration, but never exceeded 43% (700 mg/L). After four weeks, all otoliths and pectoral spines were determined marked for 700 mg/L OTC, 20% for fish immersed in 450 mg/L OTC, and 0% were marked after four weeks at the 250 mg/L OTC. Results show, channel catfish fingerlings can be successfully marked with immersion in OTC at 700 mg/L for at least 6 hours.

INTRODUCTION

Ictalurids are some of the most pursued recreational fish species in the United States (USFWS and USCB 2006). Many state agencies supplementally stock channel catfish *Ictalurus punctatus* to support put-grow and put-take fisheries (Michaletz and Dillard 1999). In Oklahoma, for example, the Oklahoma Department of Wildlife Conservation (ODWC) stocks an estimated 460,000 channel catfish annually to provide consistent fishing at waters they manage (S. Spade, ODWC, pers. commun.). One way to help quantify contribution of stocked channel catfish to these fisheries is to mark hatchery-reared fish. Some studies have marked channel catfish by fin clips (Storck and Newman 1988; Siegwarth and Johnson 1998; Odenkirk 2002) or attaching external tags (e.g., Michaletz et al. 2008), but these methods require a great deal of time and personnel to mark a mass quantity of fish. As a result, a protocol that allows for marking several thousand channel catfish at once would be desirable.

Oxytetracycline (OTC; $C_{22}H_{24}N_2O_9$), an antibacterial chemical (Rach et al. 2008), has been used to batch-mark a variety of species such as American shad *Alosa sapidisima* (Lorson and Mudrak 1987), crappie *Pomoxis* spp. (Isermann et al. 1999), and yellow perch *Perca flavescens* (Brown et al. 2002). However, only two studies report applying OTC to ictalurids, and both required handling the fish individually. Murie et al.

(2006) successfully marked pectoral spines of yellow bullheads from South Florida by injecting OTC interperitoneally. Stacell and Huffman (1994) evaluated the susceptibility to photosensitivity in channel catfish after being administered OTC interperitoneally. Therefore, the objective of this study was to evaluate the efficacy of OTC immersion at three concentrations as a way to mass-mark channel catfish fingerlings.

METHODS

We obtained approximately 550 channel catfish fingerlings (~ 25 mm) from the ODWC at Byron Fish Hatchery and placed them in three Living Streams (793 to 1,019 L; Frigid Units Inc., Toledo, OH), each equipped with refrigeration units that provide circulation and a consistent water temperature of 21° to 22° C. Fish were divided into three tanks that were randomly assigned to one of three OTC concentrations (250, 450, and 700 mg/L) and then immersed in the solution for a 6 hour duration. Tap water, treated to remove chlorine, was mixed with sufficient quantities of OTC (HCL) to create solutions of 250, 450, and 700 mg/L OTC hydrochloride. The OTC mixture was buffered to a pH of 7.0 with sodium phosphate (dibasic, Na_2HPO_4) before being added to the Living Stream treatment tank. The tank used for marking was covered using a tarp to prevent degradation of the OTC by light (Choate 1964; Trojnar 1973; Kayle 1992) and aeration was provided continually by aeration pumps. Water temperature, pH, and fish mortality were monitored every hour during the immersion period.

After immersion, fish were transferred back to their tank for monitoring and kept in floating pens sorted by treatment group. After two weeks, the fish in their pens were transferred to a larger 2,271-L flow-through tank equipped with aeration and water temperature maintained between 27 to 32°C. The pens were examined once per day to assess fish mortality. Fish were fed a diet of pellets three times a day (S. Spade, ODWC, pers. commun.).

To assess OTC mark presence, approximately ten channel catfish per trial were harvested at 1-week and 4-week post-mark, and lapilli otoliths (Long and Stewart 2010) and pectoral spines were removed. Otoliths and pectoral spines were embedded in epoxy and sectioned with a Buehler® low-speed isomet saw. Each section was sanded wet with 600 to 1,000 grit sandpaper as described by Stewart et al. (2009). Otoliths were sectioned in the transverse plane and pectoral spines were sectioned at the basal recess. The presence of an OTC mark was determined by using an epi-fluorescent compound microscope (Motic BA400T-FL, Motic Incorportation LTD, Hong Kong) equipped with a 100-W ultraviolet (Hg arc) light source and fluorescent filter (495 dichroic mirror, 470 excitation filter, and 515-nm IF barrier filter). Because growth has been shown to affect OTC mark detection (Murie et al. 2006), we compared differences in growth (mm/day) among treatments at one and four week post-mark using an analysis of variance (ANOVA).

RESULTS

The channel catfish marked in this study exhibited good survival during and after marking with total mortality less than 12% during the 4 week study (Table 1). Approximately 454 fish were immersed and all fish survived the marking period. However, two groups exhibited fairly high post-marking mortality rates. The group exposed to 250 mg/L concentration had the highest mortality rate (40%), which was nearly double the 450 mg/L group that experienced 23% mortality.

Growth (mm/day) of fish after one and four week post-mark was similar among treatment groups ($P > 0.05$, Table 1). After one week, fish averaged 25 mm in length and grew from 0.1 to 0.3 mm/day. After four weeks post-marking, fish averaged 75 mm in length and grew from 6.7 to 7.0 mm/

Table 1. Mean growth, percent mortality and percentage of otoliths and pectoral spines of channel catfish, *Ictalurus punctatus*, marked after immersion in oxytetracycline (OTC) hydrochloride solutions at three concentrations at 6 hour durations of immersion time. Percentages of fish marked were assessed 1-week and 4-weeks post-immersion. *N* is the number of fish for which each structure was assessed for OTC mark presence.

Concentration of OTC (mg/ L)	Growth (mm/ day)		Percent Mortality		Otolith				Pectoral Spine			
					1-wk		4-wk		1-wk		4-wk	
	1-wk	4-wk	0-1 wk	1-4 wk	*N*	% marked	*N*	% marked	*N*	% marked	*N*	% marked
250	0.2	6.7	5	40	10	0	10	0	10	0	10	0
450	0.3	6.9	23	8	10	0	10	20	10	0	10	20
700	0.1	7.0	12	12	7	43	10	100	10	43	10	100

day.

Overall, a total of 57 (13% of the population that was marked) channel catfish from all concentrations was checked for presence of OTC marks on otoliths and pectoral spines. After one week post-marking, no marks were observed on pectoral spines at all concentrations or otoliths marked at 250 and 450 mg / L concentration. The 700 mg / L concentration produced marks on otoliths in 43% of fish.

After four weeks post-marking, OTC marks were frequently visible on both structures depending on concentration (Table 1). No marks were observed on otoliths marked at 250 mg/L OTC hydrochloride. The 450 mg/L concentration produced marks on all otoliths and pectoral spines, but only on 20% of the individuals. The 700 mg/L concentration produced marks on both otoliths and pectoral spines.

DISCUSSION

No mortality was observed during the immersion period for any of the concentrations, which we attribute to our use of controlled water temperatures and water recirculating system to reduce waste build-up. We did have post-immersion mortality, though, and we believe this was mainly due to the increased photosensitivity of the catfish that were exposed to OTC (Stacell and Huffman 1994), because all dead fish exhibited symptoms consistent with photosensitivity (i.e., white ocular lenses and dorsal epidermal necrosis).

If the OTC itself or its ancillary changes in water chemistry (e.g., pH) contributed greatly to fish mortality, we should have observed greater losses of fish with the higher concentrations, which we did not. As a result, we feel like the post-immersion photosensitivity probably exerted the greater effect on fish survivorship. All tanks experienced some sunlight exposure post-immersion, but the treatment that exhibited the highest mortality (250 mg/L) received sunlight for a longer period of the day than the others. Studies with control fish (i.e., non-marked) would help resolve which factor(s) contributed to post-immersion mortality, but because we were confident that OTC would result in marks (Stacell and Huffman 1994, Murie et al. 2006) and we were only interested in evaluating the concentration strength needed to produce a mark on 100% of fish exposed, we did not provide for a control group in the experimental design.

The OTC marks on otoliths were visible earlier in the study than on pectoral spines, which was probably related to fish growth. Because the otolith exhibits daily growth rings (Sakaris and Irwin 2008), it is logical that this structure showed OTC sooner than pectoral spines because daily rings can be discerned on otoliths, but have yet to be validated on pectoral spines of channel catfish. After 1-week post marking, fish growth was low, but once the fish were moved to a warmer environment, their growth increased by more than double and OTC marks became highly visible on otoliths and pectoral spines. For yellow bullheads that were injected with OTC, those that did not grow failed to produce a mark (Murie et al. 2006). Overall, growth was similar among trials and therefore suggests that it had little influence on mark formation.

Mark intensity was not assessed because of bias associated with a reader's perception of mark intensity. The marks we observed were all very visible. We felt that presence / absence adequately answered our question and precluded bias associated with variation among the visual acuity of fishery biologists (e.g., color blindness), which would affect their ability to determine mark quality.

Some researchers may choose to use pectoral spines instead of otoliths because their removal is non-lethal and our findings show that OTC marks can be produced on pectoral spines under the correct circumstances. However, a major concern with pectoral spines is the erosion of annuli by the expanding central lumen which may preclude OTC verification. As a result,

studies that rely on marking channel catfish pectoral spines with OTC should be aware that marks may disappear with the lumen, although Murie et al. (2006) found OTC marks in yellow bullheads from South Florida one year post-injection.

This study is one of the first to validate the use of OTC immersion as a method to mass mark channel catfish in high densities. Yellow bullheads were successfully marked with OTC, but were handled individually (Murie et al. 2006), which is unrealistic for marking a large number of fish. Based on our findings, marking channel catfish by immersion in 700 mg/ L OTC hydrochloride for 6 hr following this methodology should result in 100% marking success with negligible mortality during the marking phase. However, this study should act as a guide to further direct future studies assessing the use of OTC to mark channel catfish. For example, it would be beneficial to examine alternative exposure times at these concentrations. In addition, negative effects caused by light exposure are of concern and preventative measures post-immersion would help decrease mortality. There is a need for a more intensive study to examine mortality caused by photosensitivity prior to implementing this as a regular method to identify hatchery reared channel catfish. Furthermore, because our study was based on one trial at each concentration, further replication by others would help ensure the validity of our results.

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