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Mark retention and fish survival associated with a low-cost marking technique for common carp *Cyprinus carpio* (Linnaeus, 1758)

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Abstract

Common carp (*Cyprinus carpio*, [Linnaeus, 1758]) have long been established in the United States and in some cases their populations can be very dense, leading biologists to expend significant effort towards reducing numbers of common carp in some populations. Estimating abundance of common carp prior to removal efforts would be useful in evaluating success of these removal efforts, but marking large numbers of fish can be expensive. Therefore, a low-cost tagging option is needed. In this study, we used tank and field experiments to determine the retention and longevity of hole-punch marks in the opercula of common carp. For the tank experiment, fish were double marked with a size-3 self-piercing tag and an operculum hole-punch mark (using a paper hole-punch tool with a hole diameter of 6.4 mm) on opposite sides of the fish. Over the entirety of the 180–day tank experiment, retention of the self-piercing tags and hole-punch marks was 100% and no marking mortality was observed. For the field experiment, 883 common carp were tagged at random in two groups, a double-marked group (*n* = 416, both self-piercing tags and hole-punch) and a single-mark group (*n* = 467, self-piercing tag). Fish were sampled monthly for 398 days. Because the length distribution sampled was bimodal, we evaluated tag retention of fish <330 mm TL (small fish, *n* = 273) and > 331 mm TL (large fish, *n* = 143), separately. Hole-punch mark retention was high for both size classes throughout most of the field experiment. For large fish, retention of hole-punch marks was 100% for the entire 398-day experiment. For small fish, retention of hole-punch marks was 100% through 184-day and remained above 93% through 328-days, but declined to 0% by day 398. Our results suggest that the operculum hole-punch mark is a valuable low-cost, long-term technique for tagging common carp.

KEYWORDS
fisheries ecology,

1 | INTRODUCTION

Common carp (*C. carpio*, [Linnaeus, 1758]) are native to Asia and Europe, and have become widely distributed across the globe as a result of intentional stockings for aquaculture and recreational angling (Carl, Weber, & Brown, 2016; Penne & Pierce, 2008; Weber & Brown, 2011; Weber, Hennen, & Brown, 2011). Since their arrival in the United States in the late 1,800's (Balon, 1995), the distribution of common carp has expanded due to its wide tolerance to temperature, salinity, and dissolved oxygen concentrations, and its ability to adapt to different habitats (Bajer & Sorensen, 2010).

Once established, common carp populations can become extremely dense (Drenner, Gallo, Edwards, Riegar, & Dibble, 1996). Through time, biologists have attempted to combat overabundant common carp populations using a variety of techniques. These techniques include water level manipulation, piscicide application, and removal by agency personnel or commercial fishing (Fritz, 1987; Neess, Helm, & Threinen, 1957; Stuart, Williams, McKenzie, & Holt, 2006; Verrill & Berry, 1995; Weier & Starr, 1950), although these efforts have had varying success. A management strategy to remove a specific percentage of the common carp population relies on an accurate estimate of population size. Estimation of population size is often done through mark-recapture methods where fish are marked using various tag types (Pine, Pollock, Hightower, Kwak, & Rice, 2003).

Although common carp are invasive in the United States, they receive limited attention from biologists because they have long been established and have limited recreational value. As such, dedicated financial resources to study this species is limited, so the purchase of a large number of tags to conduct a population estimate using mark-recapture methods is often not possible. Several tag types are typically used by fisheries biologist to mark fish, with Passive Integrated Transponder (PIT) and t-bar tags being the most popular (Bodine & Fleming, 2014; Rude, Whitledge, Phelps, & Hirst, 2011). Both tag types are relatively expensive (PIT tags - US $ 1.60–$ 2.78/tag; Biomark, Inc.; t-bar anchor tags - US $ 0.77–$ 1.70/tag, Floy Tag and Manufacturing, Inc.), and prices have increased ~140% in the 5 years since reported in Bodine and Fleming (2014), suggesting that the cost of these tags may continue to increase through time. Therefore, a cost effective, long-lasting method is necessary for marking common carp.

Fisheries biologists have commonly marked fish by hole punching fins or opercula (Allison, 1963; Gallagher & Wright, 2007; Miyakoshi & Hudo, 1999; Pine, Hightower, Coggins, Lauretta, & Pollock, 2012). Use of an operculum hole punch has been implemented to mark adult salmonids to estimate abundance of spawning fish (Gallagher & Wright, 2007; Miyakoshi & Hudo, 1999). Allison (1963) compared hole punch markings of caudal fins and opercula of 3-year-old lake trout (*Salvelinus namaycush*), [Walbaum, 1792]. However, this marking method has not been evaluated for common carp. Our objective was to determine if an operculum hole punch would serve as an effective marking method, by evaluating retention, and associated mortality of common carp during a 180-day laboratory tank experiment. Additionally, we marked common carp with an operculum hole punch to evaluate the persistence of this mark over a 398-day period in a wild population.

2 | MATERIALS AND METHODS

2.1 | Tank experiment

Common carp were collected from Thunderbird Reservoir, Oklahoma in April 2017, using boat electrofishing (pulsed DC, high voltage, 7.5 GPP, Smith Root). Once captured, they were placed in a live well and transported back to the boat ramp where they were offloaded into a hauling tank. From there carp were transported to the Oklahoma Fishery Research Laboratory in Norman, Oklahoma, where they were acclimated to laboratory conditions by mixing well water with the lake water over a 6 hr period. Once acclimated, fish were measured for total length (TL, mm) using a measuring board, and tagged while on the measuring board with an individually numbered size-3 self-piercing tag ($ 0.12 each; National Band & Tag Co.) between the opercular and sub-opercular bones on the right lateral side of the fish (Figure 1). On the opposite side (left lateral side) of the fish, the opercular bone was marked centrally in the dorsal region using a hand held paper hole-punch tool (15 sheet capacity with an hole diameter 6.4 mm). This was done by raising the operculum to ensure gills were out of the way, aligning the hole-punch tool in the center of the operculum, and squeezing to punch the hole. Common carp were then placed into a 3,032-L raceway (inside dimensions = 3.7 m × 1 m × 1 m) with slow water exchange and aeration to maintain oxygen levels of ≥6 ppm and water temperatures of 21.3°CC ± 0.9°C for the duration of the 180-day tank experiment. Carp were fed an artificial diet consisting of a size 3 floating and sinking pellet twice daily. Common carp mortalities were observed daily during feeding times. Once monthly, each common carp was evaluated for retention of the

FIGURE 1 The top photograph illustrates the tag (size 3 self-piercing tag) placement between the operculum and sub-operculum of common carp. The bottom photograph illustrates tissue growth covering a self-piercing tag on a common carp after 247-days

self-piercing tag and condition of the hole punch scar. An operculum that is considered healed looks normal, with the exception of the perfect circular shape created by the hole-punch tool. Prior to being healed completely, the tissue covering the hole is translucent, but becomes opaque when entirely healed.

2.2 | Lake experiment

Common carp were collected at Lake Carl Etling during May 2017–May 2018 using boat electrofishing. Collection to initially mark fish was conducted by sampling the entire perimeter of the lake twice over two consecutive days. A total of 883 common carp were captured and tagged during this initial effort, and fish were tagged at random in two groups, a double-marked group and a single-marked group. Double-marked fish (*N* = 416 common carp) were marked with both a size-3 self-piercing tag on the right operculum and a hole punch in the left operculum. Single-marked fish (*N* = 467 common carp) were tagged only with a size-3 self-piercing tag to determine whether false identification (seeing what appears to be a scar that does not exist) of operculum hole punch scars occurs. Following the event, common carp were resampled monthly over the 1 year period, with the exception of January 2018 when the lake was frozen. If a self-piercing tag was observed, the tag number was recorded and the opposite operculum was inspected by two personnel to determine presence of a hole-punch mark.

2.3 | Statistical analysis

Because fish size could affect tag retention, a Kolmogorov-Smirnov Test was used to determine if size structure differed between groups (individuals tagged with both self-piercing and hole-punch marks, or only self-piercing tags). Further, the length frequency histograms were visually inspected to confirm differences detected by the Kolmogorov-Smirnov (K-S) test since it becomes sensitive to large sample sizes (Neumann & Allan, 2007). All statistical analyses were conducted at a significance level of $p \le .05$.

Retention of hole-punch marks was estimated using the Kaplan-Meier survival analysis method (Kaplan & Meier, 1958). In tank trials and field samples, fish that no longer had discernable hole-punch marks were treated as a "mortality" event in the survival analysis such that the estimated "survival" was the retention rate of marks. For field-caught fish, only fish that had self-piercing tag numbers indicating they had originally been marked with a hole-punch were considered in the analysis. Additionally, the term censored refers to individuals that are not seen again (mortality) in the analysis, while uncensored refers to individuals still (survival) in the analysis (Kaplan & Meier, 1958).

Misidentification of fish having a hole-punch mark was calculated as the number of fish observed with both a hole-punch mark and self-piercing tag that indicates no hole-punch was given divided by the total number of fish with self-piercing tag indicating no hole-punch was given. Each self-piercing tag is uniquely numbered for identification purposes.

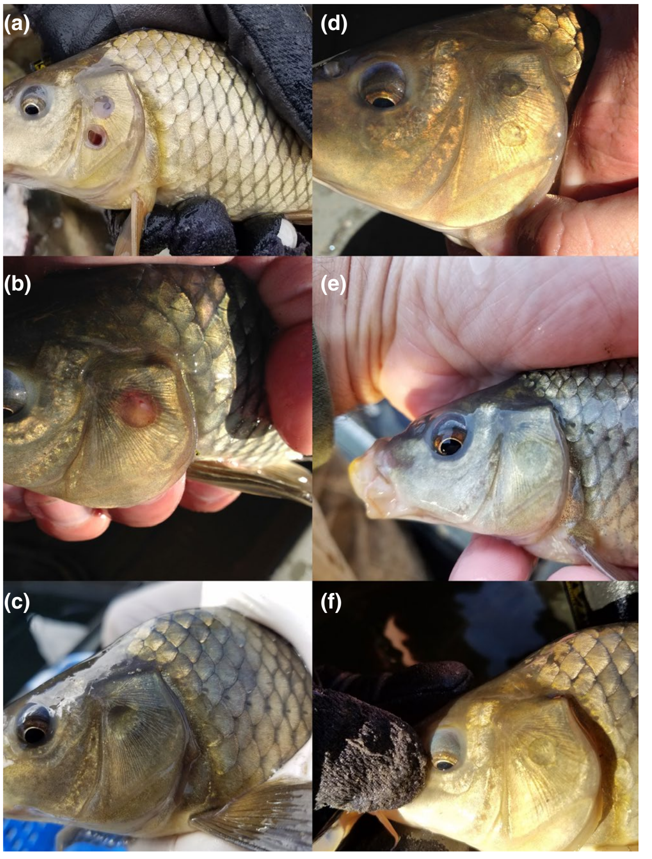

FIGURE 2 Photographs illustrating hole punch scars in opercula of common carp captured from Lake Carl Etling, Oklahoma (a = two hole punches [top hole = 59 days and bottom hole = 37 days], b = 96 days, c = 131 days, d = two hole punches [top hole = 247 days and bottom hole = 214 days], e = 328 days, and f = 398 days). Days represent the time since marking. Photographs labeled a, b and e are small fish (≤330 mm TL) and c, d, and f are large fish (≥331 mm TL)

3 | RESULTS

A total of 43 common carp (ranging 204–551 mm TL, mean = 378 mm) were collected from Lake Thunderbird for the tank trial component of the experiment. During the 180-day duration of the study, all fish retained both their self-piercing tags and identifiable hole punch mark. Additionally, no mortality was observed during the 180-day study period. The hole punch on the operculum of these common carp closed in <60 days and was completely healed within 90 days. The time required for the hole punch scar to heal in the laboratory evaluation was similar to rates observed for those marked in field component of this study (Figure 2). The mark on the opercula of larger fish (≥331 mm TL) appeared to have a depressed scar whereas the mark on small fish appeared to heal without a depression. Despite not having a depression, the hole punch scars on smaller fish were very evident as a circular scar (Figure 2).

The 883 common carp tagged in the field experiment ranged 113–494 mm TL (mean = 311 mm TL) and weighing 18–2,225 g (mean = 623 g). The length distributions between tagging treatments was not significantly different (KSa = 0.707, $p = .69$; Figure 3). The length distributions were bimodal with abundant length classes between 200–319 mm TL and 380–469 mm TL. As such, we evaluated tag retention of fish ≤ 330 mm TL (hereafter small fish, $n = 273$) and ≥331 mm TL (hereafter large fish, $n = 143$) separately by adding fish size as a treatment factor in the Kaplan-Meier survival analysis.

Hole-punch mark retention was high for both size classes throughout most of the field experiment (Table 1). Additionally, we found no false marks on individuals only tagged with self-piercing tag. For large fish, retention of hole-punch marks was 100% for the entire 398-day experiment. For small fish (≤200 mm TL), retention of hole-punch marks was 100% through day184 and remained above 93% through day 297, but then declined to zero by day 398 (Table 1). However, the last two sampling events (where retention was low) had low capture rates ($n = 3$ and $n = 2$, respectively), which also produced large confidence intervals whereby the true retention at day 328 could have been anywhere between 27.6%–100% and the confidence interval for day 367 could not be estimated because no uncensored fish remained in the experiment (i.e., no fish left were ever sampled after this date to inform what percent were still tagged).

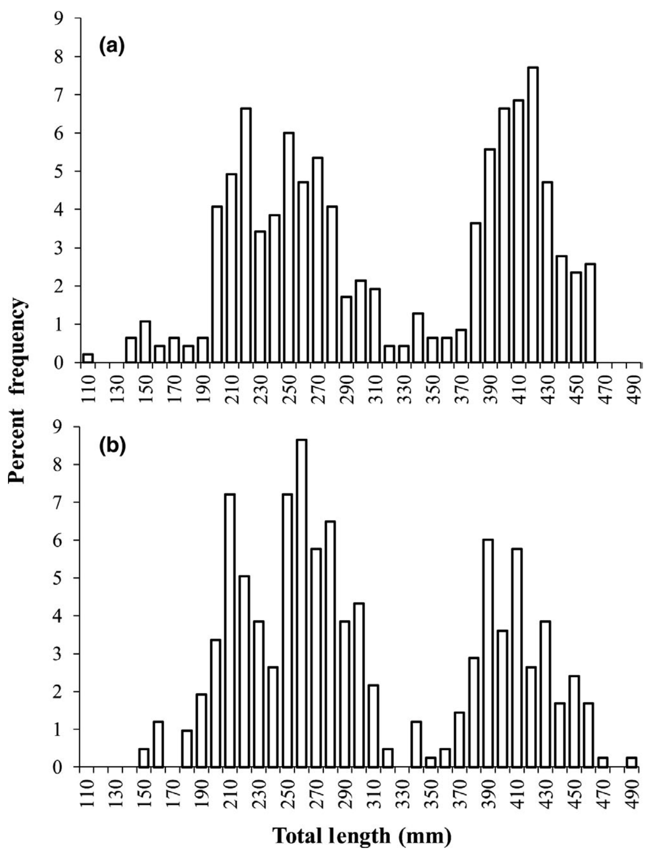

FIGURE 3 Length frequency of common carp captured and tagged using one of two tagging techniques (a = tagged only with size 3 self-piercing tag and not hole punched; b = tagged with both self-piercing tag and hole punch)

TABLE 1 Retention rates (and 95% confidence intervals; CI) of hole-punch marks on common carp sampled in field trials

	Fish ≤330 mm TL					Fish ≥331 mm TL				
Days post-marking	# of fish still in analysis	# observed without mark	# censored	Retention rate	95% CI	# of fish still in analysis	# observed without mark	# censored	Retention rate	95% CI
0	273	0	213	100.0%	(100–100%)	143	0	85	100.0%	(100–100%)
59	60	0	12	100.0%	(100–100%)	58	0	6	100.0%	(100–100%)
96	48	0	10	100.0%	(100–100%)	52	0	4	100.0%	(100–100%)
131	38	0	10	100.0%	(100–100%)	48	0	5	100.0%	(100–100%)
164	28	0	4	100.0%	(100–100%)	43	0	7	100.0%	(100–100%)
184	24	0	9	100.0%	(100–100%)	36	0	4	100.0%	(100–100%)
214	15	1	6	93.3%	(81.5–100%)	32	0	7	100.0%	(100–100%)
247	8	0	2	93.3%	(81.5–100%)	No large fish recaptured this month				
297	6	0	3	93.3%	(81.5–100%)	25	0	9	100.0%	(100–100%)
328	3	1	0	62.2%	(27.6–100%)	16	0	6	100.0%	(100–100%)
367	2	2	0	0.0%	NA	10	0	7	100.0%	(100–100%)
398	No small fish recaptured this month					3	0	3	100.0%	(100–100%)

Note: Retention rates were estimated using a Kaplan-Meier survival analysis. Censored fish were fish that were not seen again in the analysis (i.e., not recaptured after that date so their tag retention status cannot be evaluated).

Survival of small fish in the field experiment could not be calculated for the last day because no small fish were captured on that date (Table 1).

4 | DISCUSSION

To our knowledge, this is the first study to evaluate retention of an operculum mark using common carp as the test species. Retention of operculum marks was high (>93.3%) for small fish for 297 days of the evaluation. However, mark retention dropped to 0% after 297 days, but was likely affected by low sample size (three individuals). Similarly, reductions in mark retention with increased time has been observed in previous tagging studies using various tag types (ex. loop, monel, PIT and t-bar anchor tags; Basavaraju et al., 1998; Briggs, Boase, Chiotti, Hessenauer, & Wills, 2019; Diethrich & Cunjak, 2006; Hammel, Hammen, & Pegg, 2012). Operculum mark retention for large fish was 100% over the entire 398 day study. The operculum-mark retention rates we observed for small fish were comparable to rates observed for 3 year old lake trout (100% retention through 9 weeks, but decreasing to 77% by the end of 32 weeks; Allison, 1963). Allison (1963) also used an external light source to aid in operculum mark detection, which may have resulted in the high mark retention rates in that study. We did not use an external light source in this study, however this technique is recommended as it may have helped discern marks, especially for small fish after 297 days.

Fisheries biologists often look for low cost methods for tagging fish (ex. branding [hot, cold, or chemical], fin clipping, or fin hole punching; Allison, 1963; Gallagher & Wright, 2007; Miyakoshi & Hudo, 1999; Pine et al., 2012). However, branding and fin clipping are most suitable for short term fish marking studies, because healing or regeneration rates are rapid and affects the ability to detect these marks through time (Guy, Blankenship, & Nielsen, 1996). When these marking techniques are applied to common carp, Basavaraju et al. (1998) found that branding (cold or silver nitrate) was not useful for marking common carp of all sizes (10–800 g) due to low retention rates. Further, fin clipping (pectoral, pelvic, and upper or lower caudal fin) small common carp (10–25 g) was not suitable due to rapid fin regeneration, however fin clip retention of large common carp (600–800 g) was high (96.7% for 129 days; Basavaraju et al., 1998). Compared to these low cost marking methods, common carp marked with operculum hole punches had higher retention rates (93%–100%; 200–494 mm TL) over a longer time period (297 days). Further, the operculum hole punch mark is less invasive when compared to complete removal of pectoral and pelvic fins, or clipping of the entire upper or lower caudal fin lobes of common carp, which was done in prior common carp tagging evaluations (Basavaraju et al., 1998).

The operculum is a robust, boney plate that protects the gills of fishes (Farag, Wally, Daghash, & Ibrahim, 2014), and has commonly been used to estimate ages of common carp (Vilizzi, 2018). The ability to estimate ages from opercula of common carp suggests that calcium is deposited daily on this structure, forming an annulus as fish growth slows during winter. We hypothesize that hole punch mark retention was lower in small, young common carp (≤300 mm TL) because the bony tissue needed to repair the thin operculum of these fish is produced rapidly as these fish grow. Conversely, the hole in the opercula of larger, adult fish has removed a large amount of layered tissue and takes much longer for these relatively slower-growing fish to fill the thicker void. This theory is supported by the hole punch scar in larger fish appearing depressed as represented in Figure 2. Generally, growth slows with increasing age, therefore taking a longer time for the opercular bone on large fish to become indiscernible from unmarked bones. Our results suggest that mark retention longevity of opercular scars on large fish may surpass 398 days.

Our results suggest that the operculum hole punch is a valuable technique for marking common carp. Further, we observed high mark retention rates for 297 days for both length classes during the field trial component of this study, and we documented no abnormalities or mortality associated with the hole punch mark during the tank trail. A shortcoming of this marking technique is the inability to differentiate individual fish, however fish can be marked using a variety of hole combinations (in both opercula using multiple hole punches) or hole punches in different locations making it useful for distinguishing different batches of fish. This technique provides fisheries biologists with a low-cost, long-term marking option, which will aid in the management of common carp populations, particularly where resources to study this species are limited. Additionally, it appears that this marking technique can be applied to other fish species and populations (Allison, 1963, this study) and our results suggest it may be best suited for slower growing fish species or larger fish, but should be evaluated on a case-by-case basis prior to widespread use.

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DATA AVAILABILITY STATEMENT

No Data Available.

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