Evaluation of Hole-Punching Operculums as a Marking Technique in Centrarchids

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Abstract: Mark-recapture studies provide important information about fisheries. There are many ways to mark or tag fish, but some populations of fish, like Largemouth Bass (Micropterus salmoides) and sunfish (*Lepomis* spp.), will require a large number of fish to be tagged. The cost of tags could be prohibitive to these studies. Lower-cost methods help to reduce this barrier. This study was split into a lab and field trial to investigate the applicability of operculum hole-punching as a viable, low-cost option for Centrarchid mark-recapture studies. In the lab, 63 Largemouth Bass and 105 sunfish were collected and marked with a self-piercing tag on their left operculum. Of those, 33 bass and 53 sunfish were marked with a 6.4mm paper hole-punch tool on their right operculum then fish were held in pools to test growth rates, mortality, and longevity. Results showed no significant difference between hole-punch and control fish for growth or mortality. Next, 60 Largemouth Bass and 328 sunfish were collected from a 6-acre pond. Fish were marked with a hole-punch and a fin clip. Hole-punch and fin clip locations varied based on the date collected. In both studies, hole-punch closure rate was observed in 25% increments. Overall, hole-punches closed faster in the lab study, suggesting environment can affect growth rates. Also, Largemouth Bass marks closed faster than sunfish showing a species-specific difference. Approximate operculum hole-punches closure rates take 26 days to close in Largemouth Bass and 164 days in sunfish. Our results suggest operculum hole-punching is a viable low-cost method for marking fish.

Introduction

Mark-recapture studies provide information about fisheries population dynamics and vital rates. These studies allow for the estimation of metrics such as mortality, population abundance, recruitment, and stocking success (Conover and Sheehan 1999). To estimate these parameters via mark-recapture, fish are marked or tagged externally (e.g., fin clipping, strap tags, floy tags) or internally (e.g., passive integrated transponder [PIT] tags, oxytetracycline [chemical marking of hard structures; Parker et al. 1990]). Tag cost can range anywhere from

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~$2.11 (PIT Tags) to ~$0.29 (straps tags) per tag (Nathanael Hull, Oklahoma Department of Wildlife Conservation, unpublished data). In certain populations (e.g., high abundance) a large number of fish will likely need to be tagged, potentially making the cost of tags prohibitive (Schneider 1998). In these instances, lower-cost methods (e.g., marking via fin clipping or punching) are commonly used; however, this generally does not allow for the recognition of individuals (Wagner et al. 2009, McFarlane et al. 1990). Date specific (e.g., left pelvic clip day one, right pelvic day two) or batch marks (e.g., all fish get right pelvic clip) are commonly applied in these instances.

Though generally not an issue for batch marking, date specific marking often limits the number of possible mark-recapture samples. For example, Everhart et al (1975) found only 10 possible date specific marks when two paired fins were used. One common alternative to fin clipping is punching holes in fins. Fin hole punches have been used since 1896 (Mcfarlane et al. 1990). Punching holes leaves a smaller mark and likely induces less stress on the fish. However, the mark is smaller making it harder to identify and allowing it to regenerate faster (Murphy and Willis 1996). Hole-punching operculums is another method that has received more attention in recent years. Operculum hole punches have been applied to Lake Trout *Salvelinus namaycush* (Allison 1963), Masu Salmon *Oncorhynchus masou* (Miyakoshi and Kudo 1999), Common Carp *Cyprinus carpio* (Snow et al. 2020), and Rainbow Trout *O. mykiss* (Rosburg et al. 2022). The retention of operculum marks has yet to be studied on Centrarchids.

Centrarchids are high-value sportfish (e.g., Largemouth Bass *Micropterus salmoides*) or commonly targeted (e.g., Bluegill *Lepomis macrochirus*) throughout their range (Quinn and Paukert 2009). Different tagging and marking methods have been studied using Centrarchids. Largemouth Bass and Bluegill have been PIT tagged (Siepker et al. 2012, Kaemingk et al. 2011), marked with injectable fluorescent tags, implant elastomers (Catalano et al. 2001), and t-bar anchor tagged (Tranquilli and Childers 1982, Parsons and Reed 1998), to name a few. Having additional low-cost marking strategies for these species would benefit fisheries managers, especially given they can occur in high abundances (Jennings 1997). Therefore, the goal of our study was to investigate the applicability of operculum hole-punching as a viable option for Centrarchid mark-recapture studies. Our specific objectives were to (1) estimate the longevity of operculum hole-punches for Centrarchids and (2) determine if hole-punch longevity varied between species group (specifically Largemouth Bass *Micropterus salmonids* and *Lepomis* spp.) or lab and field settings.

Methods

Lab Trial

Twenty-one Bluegill were collected from Sparks Lake, Oklahoma on March 31, 2021. Eighty-four Bluegill, Redear Sunfish *Lepomis microlophus*, Green Sunfish *L. cyanellus*, and hybrid sunfish *Lepomis* spp. were collected from Dahlgren Lake on April 1-2, 2021. Additionally, sixty-three Largemouth Bass were collected from Konawa Lake, OK on April 6, 2021. Fish were collected using boat-mounted electrofishing regulated via a Smith-Root 7.5 generator-powered pulsator (settings: 120 DC, 120 & 60 pulses per second, 20-40 % of power). Only fish ≥ 127 mm total length (TL) were retained for use in the lab study. Fishes were held in the live well while on the boat, offloaded into a hauling tank at the ramp, and then transported to the Oklahoma Fishery Research Laboratory in Norman, OK.

At the lab, fish were measured to the nearest 1-mm TL and a style 1005-3 self-piercing tag (National Band & Tag Company) was placed on their left operculum so fish could be uniquely identified. A 6.4 mm (diameter) handheld paper single-hole punch tool was used to mark fishes in the center of the operculum. Fifty-three *Lepomis* spp. received a hole punch in their right operculum and 52 were used as controls. Thirty-three Largemouth Bass were hole-punched on their right operculum and 30 were used as unmarked controls. After marking, fish were placed in holding pools (4.6 m diameter, 1.2 m deep; Intex Recreation). Holding pools were aerated and received fresh well water. Bass and sunfish were placed into separate pools, with the exception of three Largemouth Bass < 230 mm TL that were placed with the *Lepomis* spp. Dissolved oxygen and water temperature were monitored regularly via a handheld YSI Pro 1020 (Xylem Inc.). Fish were fed once daily with either a size 3 floating pellet (Purina) or Trophy Fish Feed Multispecies formula (Sportsman's Choice). Minnows were placed into both pools for additional forage. In addition to minnows, Largemouth Bass received *Lepomis* spp. < 120 mm TL for forage.

Pools were sampled intermittently to check the progression of the hole closure (Figure 1). Largemouth Bass were sampled four times over six weeks and *Lepomis* spp. were sampled five times over seven weeks. During sampling, fishes were enclosed in a seine to reduce capture area. Fishes were then netted out using a short-handled dip net and placed into 757-L holding tanks. Fishes were examined to see if they had a hole-punch, how much of it had filled in (%), and their self-piercing tag was recorded. If their self-piercing tag had fallen out TL was recorded to try and match individuals based on

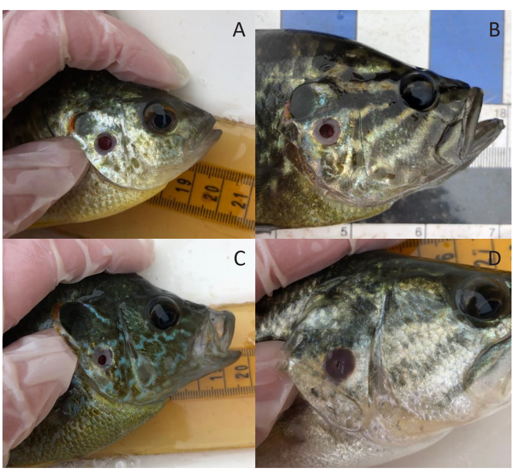

Figure 1. Photographs showing hole-punch closure percentages in the operculum of sunfish and Largemouth Bass from the lab study. (A – 75% closure, B-50% closure, C-25% closure, D-5% closure).

size. For *Lepomis* spp., species was also noted. Any mortalities were collected from pools and assigned to either hole-punched or control fish groups. At the end of the study, TLs were recorded for all fishes to estimate growth rates.

Field Trial

Largemouth Bass and sunfish were sampled via boat-mounted electrofishing regulated by a Smith-Root 5.0 generator-powered pulsator (settings: High Range, DC, 120 pulses per second, 20-60 % of power) at Northeast Lions Park Pond, a 6-acre pond in Norman, OK. Six samples were taken between 9/21/22 and 4/6/23. Fish ≥ 127 mm TL were measured (mm), weighed (g), and hole-punched in the same manner described prior for the lab trial. Fishes were marked with unique pelvic fin clip and hole-punch combinations during each sampling event so marking day could be estimated (Table 1). All recaptures were weighed and measured, and we noted the side tagged and fin clipped. We observed the progression of the hole punch closing in 25% increments, with 100% being completely open to 0% being fully closed.

Data Analysis

To determine if hole punching resulted in reduced growth, we estimated growth rates for each fish that survived until the end of the lab trial by subtracting their final TL from their initial TL. We then compared growth rates using a two-sample Kolmogorov–Smirnov (K–S) test ($\alpha = 0.05$, Kolmogorov 1933, Smirnov 1939). To determine the amount of similarity between growth rates for control and marked fish we estimated distributional overlap ($\hat{\eta}$; Pastore and Calcagnì 2019) via the "boot.overlap()" function from the "overlapping" package (Pastore 2020). We used because it is a distribution-free metric that allows for estimates of relative similarity between distributions. We derived means and 95% confidence intervals (CIs) for by bootstrapping the comparison 1,000 times. We interpreted based on its relationship with Cohen's d (Cohen 1988); meaning $\hat{\eta} = 0.20$ indicates a small distributional overlap, $\hat{\eta} = 0.50$ indicates moderate distributional overlap, and $\hat{\eta} = 0.80$ indicates large distributional overlap (Pastore 2020).

Table 1. Dates when hole-punching samples were taken from Northeast Lions Park Pond, OK. Included are the side of operculum that was punched and the side of the pelvic fin that was clipped during each sampling event. During the last two sampling events, only recaptures were collected.

Date	Side Operculum Punched	Side Fin Clipped
9/21/2022	Left	Left
9/23/2022	Left	Right
10/7/2022	Right	Left
10/27/2022	Right	Right
11/17/2022	Recaptures Only	Recaptures Only
4/6/2023	Recaptures Only	Recaptures Only

To determine if hole-punching resulted in significantly ($\alpha = 0.05$) higher mortality a χ^2-test was used to compare survival and mortality between hole -punched and control fish. Strength of association was estimated for the χ^2-test using Cramér's V statistic (Acock and Stavig 1979). Cramér's V was interpreted based on its association with Cohen's ω statistic when a table has two rows (Choen 1988). Meaning $V < 0.30$ indicates low association, $V < 0.50$ indicates medium association, and $V \geq 0.50$ indicates strong association between groups. We derived 95% CIs for Cramér's V using 1,000 bootstrap replicates.

Total lengths of marked Largemouth Bass and sunfish from lab and field trial were compared using a K–S test ($\alpha = 0.05$). Estimates of (mean and 95% CI) were also obtained for both species TL comparisons. The procedure for obtaining estimates was the same as described prior for lab growth rate comparisons.

To determine if mark-retention was similar between lab and field trials a binomial distributed generalized linear model with a probit link was fit via the "glm()" function within R Statistical Computing Platform version 4.3.0 (R Core Team 2023). The global model included predictive parameters day (number of days since marking), system type (i.e., lab vs field), and species (i.e., *Lepomis* spp. vs Largemouth Bass). Also included in the global model were two- and three-way interactions between predictors. Prior to analysis Pearson's product-moment correlations were compared for all predictive variables to ensure no strong correlations (i.e., $r > |0.70|$, Akoglu 2018) existed.

A backwards selection process was used to determine which variables and interactions were most important in describing mark retention (James et al. 2013). Insignificant parameters ($p > 0.05$) were removed starting with the highest order interaction (i.e., three-way interaction), then second-order interactions, followed by main effects if necessary. Fit of the final model was assessed via residual, scale location, and leverage plots. The final model (i.e., model with only significant predictors or interactions) was compared to all prior models using a likelihood ratio test ($\alpha = 0.05$) via the "lrtest()" function from the "lmtest" package (Hothorn et al. 2022). This allowed us to confirm that the backwards selection process (i.e., removal of insignificant predictors) did not result in a model with poorer relative fit to the data (Achim and Torsten 2002). Predictions from the final model were used to estimate the longevity of hole punch marks for Centrarchids.

Results

Lab sample size consisted of 33 Largemouth Bass and 53 sunfish, which were recaptured 127 and 225 times, respectively (Table 2). During lab trials 8 control and 8 hole-punched sunfish died, and no bass mortality was observed. In the field trial, 60 Largemouth Bass and 328 sunfish were hole-punched during fall and spring months. Of those, 16 Largemouth Bass and 37 sunfish were recaptured in the fall, and 11 bass and 12

Table 2. Total number of marked fish and number of recaptures for *Lepomis* species and Largemouth Bass (*Micropterus salmoides*) from the lab and Northeast Lions Park Pond (Field).

System	Species	Marked	Recaptures
Lab	*Mciropterus salmoides*	33	127
Lab	*Lepomis* spp.	53	225
Field	*Mciropterus salmoides*	60	27
Field	*Lepomis* spp.	328	49

sunfish were recaptured in the spring. The hole-punches from field trial fish were fully filled in but observable in spring samples.

Hole punching did not appear to increase mortality or decrease growth of bass or sunfish based on lab trials. The chi-squared test showed no significant difference in mortality between hole-punched and control fish ($\chi^2 = 0.47$, $p = 0.49$). Cramér's V suggested low association between hole punching and mortality (mean [95% CI] = 0.08 [0.01-0.22]). Confirming hole punching did not increase fish mortality. Additionally, hole punches did not affect fish growth rates. The K-S test suggested growth was not significantly different between hole-punched and control fish (D = 0.20, p = 0.29). Estimates of $\hat{\eta}$ suggested moderate-to-high similarity between both groups (mean [95% CI] = 0.67 [0.49-0.82]). Confirming hole punching did not decrease fish growth.

We compared TLs of fish studied between lab and field trials. The total length of bass in the lab study ranged from 180-540mm and from the field component ranged 127-585mm. KS test for Largemouth Bass suggested there was a significant difference between TLs for fish marked in the lab and field trials (D = 0.32, $p < 0.05$). Estimates of $\hat{\eta}$ suggested moderate-to-low similarity between both groups (mean [95% CI] = 0.52 [0.35-0.71]). Suggesting that there was at least a moderate statistical difference between TLs for Largemouth Bass in the lab and field setting. This was likely due to smaller Largemouth Bass being marked during the field trial. The total length of sunfish in the lab study ranged from 127-221mm and from 127-215mm in the field study. The K–S test for sunfish suggested there was no significant difference between TLs for fish marked in the lab and field trials (D = 0.18, $p > 0.05$). Estimates of $\hat{\eta}$ suggested moderate-to-high similarity between both groups (mean [95% CI] = 0.71 [0.60-0.81]). This suggests that sunfish from both the lab and field trial were similar in size.

All predictor variables appeared to be important in explaining mark closure phenomena. Person's product-moment correlations suggested none of our predictors were strongly correlated (r range = -0.07 – 0.57). The final model from our backward selection process included additive effects between day, system type, and species predictors along with interaction between day and species predictors and day and system predictors (Table 3). Diagnostic plots suggested adequate model fit for the final model. The likelihood-ratio test comparing the final model to prior models from the backward selection process suggested removal of non-significant interactions did not significantly decrease model fit to the data (χ^2 range = 0.25 to 1.90, all $p > 0.05$). Two-way interactions were plotted using the "ggplot2" package to determine the effect of day and species and day and system on mark retention (Wickham 2016). Interpretation of the first interaction suggested that mark retention had an inverse relationship with days since marking lasted longer in bass compared to sunfish (Figure 2). Interpretation of the second interaction indicates that for both bass and sunfish marks were retained longer in

Table 3. Mean and standard error (SE) estimates for each parameter from the final binomial model with a probit link function obtained via backward selection. Parameters include the intercept, lab or field (System), days since marking (Day), and *Lepomis* species or Largemouth Bass (Species). Included are z-values and resulting p-values for each parameter.

Parameter	Mean	SE	z-value	p-value
Intercept	5.10	0.88	5.81	<0.05
System	-4.15	0.61	-6.84	<0.05
Day	-0.26	0.05	-5.88	<0.05
Species	-1.22	0.89	-1.37	0.17
Day × Species	0.09	0.05	2.04	<0.05
Day × System	0.17	0.03	6.39	<0.05

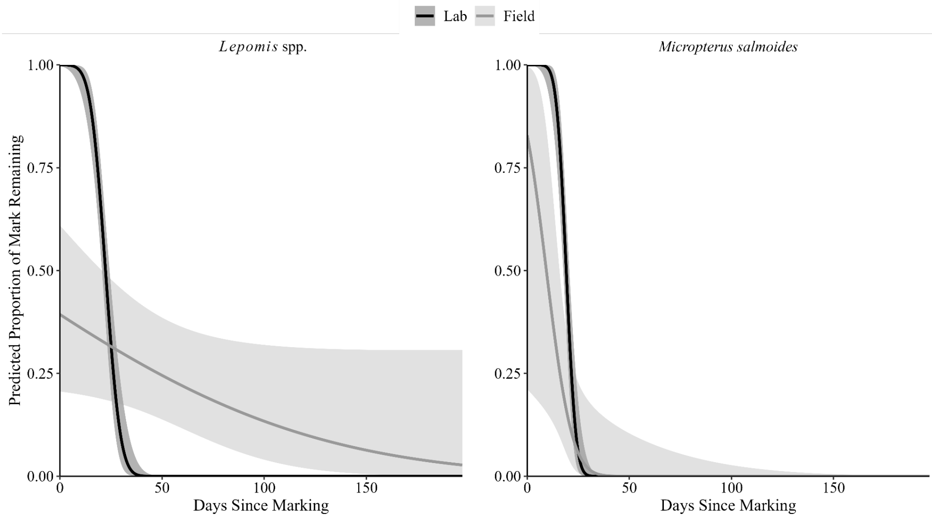

Figure 2 . Predicted proportion of mark remaining from the final probit regression model plotted across days since marking for Lepomis species and Largemouth Bass (*Mciropterus salmoides*) from lab and Northeast Lions Park Pond (Field) trials.

the field environment than lab trials (Figure 2). This suggests that mark retention estimates will vary based on species specific and environmental phenomena.

Hole punch closure rate estimates varied. Estimates at 75% and 50% open for bass suggest that marks close more rapidly in the lab setting (Figure 3). The estimates for 25% and 5% open suggested similar proportions of mark remaining in both lab and pond settings for bass. In sunfish, the 75% and 50% marks closed sooner in the pond setting than lab trials (Figure 3). Estimates for 25% and 5% open suggest that marks closed sooner in lab trials than in the pond environment. This suggests that hole punch retention measured in the field generally lasted longer than seen in lab trials, though the magnitude of this difference varied by species.

Discussion

Prior investigations into operculum hole-punch mark retentions have suggested marks remain open for < 60 (Common Carp; Snow et al. 2020) to 99 days (Rainbow Trout; Rosburg et al. 2022). Our results suggested operculum-hole punches could be reasonably expected (based on 5% open estimate) to last 26 days (95% CI = 23-29 days) for Largemouth bass and 164 days (95% CI = 93 – 174 days) for *Lepomis* spp., based on field estimates. Snow et al. (2020) showed operculum-hole punch retention was greater for Common Carp > 330 mm TL relative to those that were smaller. These results appear contradictory to our findings as *Lepomis* spp. were generally smaller than Largemouth Bass used in our study yet retained marks for a longer period. However, this is likely the result of differences in hole-punch retention between species or different definitions of hole-punch retention. Given variation in hole-punch retention present between species the literature (e.g., Allison 1963; Snow et al. 2020; Rosburg et al. 2022) it is likely that there is a difference between *Lepomis* spp. and Largemouth Bass was not due to size, but instead due to species specific differences. Furthermore, Snow et al. (2020) counted filled but still visible hole-punches as retentions as opposed to our classification of the hole punch still being open. Miyakoshi and Kudo (1999) noted that hole-punch regeneration did not affect their ability to discern marked fish, though no data were provided. No statistical analyses were performed regarding regenerated but visible punches due to our inability to discern hole-punch misclassifications. Despite this, we did note that marks could still be visually observed and that a void in the opercle bone was easily distinguished when backlighted (see Allison

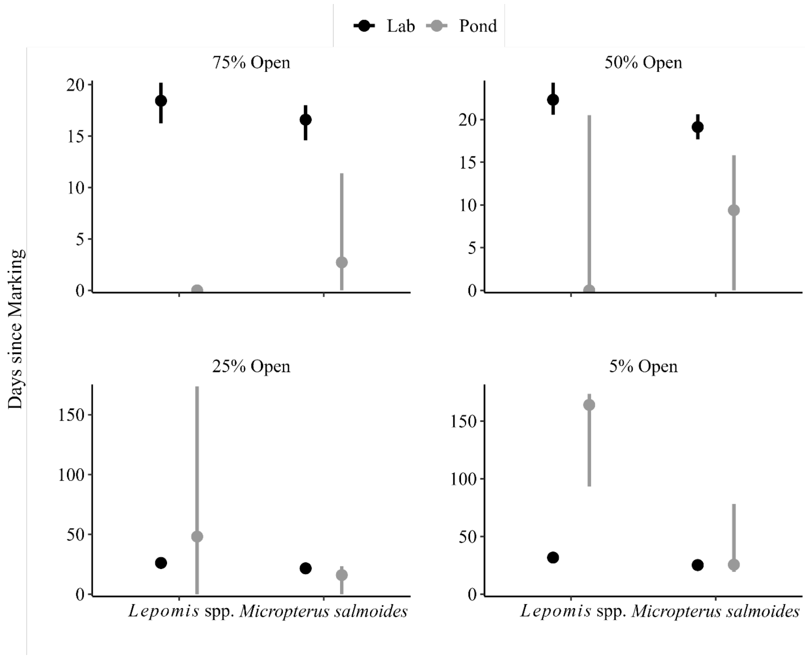

Figure 3. Closure plots showing the estimated number of days since marking at which 75, 50, 25, 5 % of the hole punch is predicted to be remaining for Lepomis species and Largemouth Bass (*Micropterus salmoides*) in lab and Northeast Lions Park Pond (Field) settings based on the final probit regression. Circles represent the mean number of days since marking and lines represent 95% confidence intervals for those day estimates.

1963). Further investigation into the ability of individuals to discern a closed hole-punch mark is needed.

Marks from hole punching operculum appeared to have different retention rates based on environment (i.e., lab vs field) and species (i.e., Largemouth Bass vs *Lepomis* spp.). In the lab, the average mark closure time for hole punches was approximately 30 days for both species. Hole-punch marks appeared to close slower in the field, though this was more extreme for *Lepomis* spp. than for Largemouth Bass. This agrees with prior studies on tags that suggested lab settings may underestimate the retention rates (Dieterman and Hoxmeier 2009). Given that a large number of variables influence tag- and mark-retention (e.g., location, fish size; Acolas et al. 2007; Pine et al. 2012; Snow et al. 2020) further field-based research into operculum hole-punch mark retention should be conducted. Potential areas for future research include Centrarchids of different sizes (e.g., < 127 mm TL), different high-abundance species (e.g., White Bass *Morone chrysops*), or different environments (i.e., different systems).

It is important to note that we are not suggesting lab-based studies are wholly unuseful for investigation into tagging or marking phenomena. Despite recent questions into their applicability (e.g., Dieterman and Hoxmeier 2009; this paper), lab-based studies are not without their merits. For example, effects of marks or tags on growth and survival are easily estimated from lab studies (e.g., Dewey and Zigler 1996; Malone et al. 1999; this study). Lab-based studies may also be preferable when investigating retention of novel tags such as pop-up satellite archival tags (Naisbett-Jones et al. 2023) or novel tag placements (e.g., operculum modified Carlin dangler tags, Montague and Shoup 2022). To the best of our knowledge, the only alternative to those worried about the validity of lab-based retention estimates are field based enclosures (e.g., net pens). Field based enclosures have been used prior to estimate tag retention (Scholten et al. 2002), along with tagging effects on growth and survival of fishes (FitzGerald et al. 2004; Dembkowski et al. 2018). However, it is unclear if field-based enclosures mimic the retention phenomenon of they systems they are placed within. Further study into the applicability of field-enclosure estimates and comparison to field estimates from non-enclosed fish is likely needed.

Our results suggest that hole-punching operculum in Largemouth Bass and *Lepomis* spp. is a viable technique for mark-recapture studies. Current estimates of retention, based on hole-punch openness, suggest this technique is best suited for closed-system techniques such as Schnabel and Lincoln-Peterson models (Pine et al. 2012). Operculum hole-punching when paired with fin clips allows for date-specific marks and can used estimates of more complex closed mark-recapture models (e.g., Otis et al. 1978; Huggins 1991). If future studies are able to discern the period of time that closed hole-punch marks are identifiable in Largemouth Bass and *Lepomis* spp. (e.g., Snow et al. 2020), it is possible that open-population mark-recapture models (e.g., Jolly-Seber; Pine et al. 2012) may be estimated with this technique. Our results suggest operculum hole-punching is a viable low-cost methods for marking fish. Investigations such as these help to reduce financial barriers for mark-recapture studies.

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