

**Evaluation of Tagging Mortality and Retention in Juvenile Humpback Chub  
Bonytail Chub as a Surrogate Species**



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## OBJECTIVES

The purpose of this study was to evaluate the utility of several tagging techniques for use in tagging endangered humpback chub in the Little Colorado River. The tags or marks used in this study were PIT tags, fin clips, elastomer dye injection, and injection of India ink. PIT tags are used for the long-term identification of individual chub, while the other three marks are useful as short-term batch marks for mark/recapture studies and estimation of PIT-tag loss in the field.

## METHODS

Bonytail chub were delivered to Bubbling Ponds Fish Hatchery, Cornville, AZ, from Wahweap National Fish Hatchery, Utah, on June 6, 2002. All fish ( $N > 1000$ ) were placed in a single 1900 L outdoor holding tank at Bubbling Ponds Hatchery. The tank was aerated, and received water from an artesian spring (110 ppm  $\text{CaCO}_3$ , 18.5 °C, pH 7.6). Fish were fed daily with trout pellets available from Bubbling Ponds Hatchery.

**PIT-tagging**—On June 10, 180 fish were injected with PIT tags using a hypodermic syringe, and placed into a second holding tank. Fish were not fed prior to tagging on this date. Nine biologists injected 20 PIT-tags each, following techniques typically used in the field. Each person was filmed briefly while injecting PIT-tags, using a digital video camera. In general, tags were injected in an anterior direction from a point posterior to the pelvic girdle. Each fish was measured (TL, FL), weighed (g), and received a dorsal fin punch (using a leather punch) or the dorsal fin was clipped with scissors. One hundred fish were also weighed, measured, received a dorsal fin clip, and then moved to a third holding tank, but not PIT-tagged (control). Mortality and tag loss were monitored daily, and PIT tag and dorsal fin clip/punch retention was evaluated on July 9-11, and on September 11, 2002.

On August 13, 2002, an additional 120 fish were injected with PIT-tags after being fed *ad libitum* overnight, to simulate field conditions when fish are collected in baited hoop nets. None of these fish received a dorsal fin clip or punch, but 60 of the 120 fish were injected with PIT tags after the PIT-tag hypodermic syringe had been dipped in India ink. No control group was established for this group of tagged fish. Mortality was monitored daily, and PIT tag retention, India ink retention at the injection site, and tagging scar retention were evaluated on September 11, 2002.

**Fin-clipping**—On June 10, 2002, 100 fish received a left pelvic fin clip, 100 fish received an upper caudal fin clip, and 100 fish had both the left pelvic fin and the upper caudal lobe clipped. Each fish was measured (TL, FL), and weighed (g). The control

group for fin-clipped fish consisted of 100 fish that were weighed and measured, but received no fin clips. Mark retention was evaluated on July 11, 2002.

**Elastomer dye/ India ink injection**—On August 13, 2002, 25 fish were injected with India ink (black) at the base of the dorsal fin, 25 fish were injected with elastomer dye (orange) at the base of the dorsal fin, and 25 fish were injected with elastomer dye on the dorsal surface of the head, between the eyes. Marked fish (N=75) had previously been PIT-tagged in June, so it was possible to evaluate individual mark retention, as well as evaluate false positives (as fish were returned to the tank of PIT-tagged fish after receiving elastomer or dye injection). No control group was established for the marked fish. Mark retention was evaluated on September 11, 2002.

## **RESULTS and DISCUSSION**

Mean lengths and weights of all fish used in this study are summarized in Table 1. Fish remained healthy throughout the experiment, and mortality was observed only in fish that had been PIT-tagged (below).

**PIT-tagging**—PIT-tag mortality was low for fish tagged in June (1.1%). However, fish that were fed overnight and then PIT-tagged (August) had much higher mortality (15%). No mortality was observed in the control group. All PIT-tag mortalities were immediately preserved in 10% formalin, and necropsies were performed on September 11. Almost all PIT-tag mortalities appeared to have died as a result of perforation of the intestine during PIT-tag insertion. The PIT-tag in many of the dead fish was located anterior to the pectoral fin insertion, indicating that the tag had either moved after insertion or had been injected deeper into the fish's body cavity than was necessary. Overnight feeding of the chub prior to tagging (August) may have contributed to PIT-tag mortality, as perforation of the intestine would have been more likely in fish whose intestines were full of food.

Since 10 out of a total of 18 PIT-tag mortalities in August were tagged by the same person (50% mortality for the one tagger), we recommend that a training/discussion session be held among biologist who will be PIT-tagging humpback chub in the Little Colorado River. This training session will serve to refine tagging methods used in the field, and will help ensure that a standard tagging protocol is followed.

PIT-tag retention in June was high (96.6%). No further tag loss was recorded in these fish between June and September. PIT-tag retention in August, however, was lower (92.2%). It is possible that apparent tag retention in August was lower due to the use of a PIT-tag scanner with a faulty battery/power supply. This possibility will be evaluated in October or November 2002, when fish will again be scanned.

The dorsal fin clip/punch used as a secondary mark on fish PIT-tagged in June was visible on only 83.9% of the fish in July, and on only 20.8% of the fish in September. As such, this fin clip does not appear to be an adequate secondary mark. In contrast, fish that were PIT-tagged with a tag and syringe dipped in black India ink (August) retained this secondary mark quite well (96.6% retention) for at least one month (evaluated on September 11). Only 40.8% of these fish, however, retained a visible tag scar in September. Therefore, it appears that dipping the PIT-tag needle and tag into black India ink prior to tag insertion will serve adequately as a secondary mark for evaluating tag loss in the field.

***Fin-clipping***—Visibility of fin clips (left pelvic, upper caudal, or both) was recorded approximately one month after marking fish. On July 11, 95 fish appeared to have a left pelvic fin clip, 97 fish appeared to have an upper caudal fin clip, and 103 fish appeared to have both fin clips. Three fish had no visible fin clip, and two of the fish escaped back into the holding tank before they could be examined. Thus, most fish (95.3%) were correctly classified. This percentage, however, is considerably inflated over what would be observed in the field, because all fish that were re-evaluated in the holding tank were known to have received one or both fin clips in June. This level of certainty could not exist under field conditions. Upon examination, it appeared that retention and correct classification of fin clips as secondary marks could have been improved if the entire fin had been removed. Classification of fin-clipped fish in this study was hampered by the fact that many of the fish appeared to have had only half or a quarter of the fin removed, and most of the fin had already regrown prior to being re-examined. Thus, the utility of this secondary mark is highly dependent upon the extent to which the clipped fin is removed.

***Elastomer dye/ India ink injection***—Retention of elastomer dye below the dorsal fin (24/25, 96%) and between the eyes on the head (23/25, 92%) was similar to mark retention for clipped fins. This mark, however, is highly visible and can therefore be quickly identified in the field. Elastomer dye is also likely to be retained much longer than fin clip marks. Fish that were tagged with elastomer dye will be re-evaluated in October, 2002, to determine tag retention three months after receiving the dye injection. Retention of India ink injected below the dorsal fin (15/25, 60%) was quite low in comparison to fin clips and elastomer dye injection. Thus, ink injection does not appear to be a viable tagging technique.

## RECOMMENDATIONS

- Use PIT tag needles dipped in India ink as a secondary mark for field studies of tag retention. This method appears to work well, at least as a short term mark. Further studies of marked fish at the hatchery should evaluate it's utility as a mark for longer time periods.
- Use left pelvic fin clips as a mark in the field for fish too small to PIT tag.
- Evaluate use of elastomer dye in the field as a secondary mark for fish too small to PIT tag.
- Further evaluate specific tagging methods and develop a training program for workers who will PIT tag fish in the field.

Table 1. Mean total length (TL, mm), fork length (FL, mm) and weight (W, g) for bonytail chub PIT-tagged, fin-clipped, and injected with India ink and elastomer dye at Bubbling Ponds Hatchery.

Sample Date	Group							
	PIT tag, June 10	PIT tag control, June 10	Fin clip, June 10	Fin clip control, June 10	PIT tag, August 13	Ink injection, dorsal fin	Dye injection, dorsal fin	Dye injection, head
Jun-02	TL	103.6 (0.79)	104.5 (1.09)	102.4 (0.61)	101.8 (1.07)			
	FL	90.7 (0.68)	91.6 (0.96)	90.3 (0.55)	89.7 (0.96)			
	W	8.3 (0.20)	7.9 (0.28)	8.0 (0.15)	8.9 (0.91)			
Jul-02	TL	108.3 (0.78)	109.6 (1.08)					
	FL	96.3 (0.69)	98.0 (0.94)					
	W	9.7 (0.22)	10.4 (0.34)					
Aug-02	TL				109.7 (1.11)	106.7 (1.92)	109.9 (1.48)	106.1 (2.09)
	FL				98.0 (0.98)	96.0 (1.73)	99.1 (1.36)	95.1 (1.78)
	W				11.6 (0.38)	9.9 (0.50)	10.1 (0.39)	9.1 (0.59)