Rapid Placement and Retention of Passive Integrated Transponder (PIT) Tags in Grass Carp

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Abstract: Passive integrated transponder (PIT) tags were placed in 15,344 triploid grass carp (*Ctenopharyngodon idella*) (200–280 mm total length) and length, weight, and tag-code data recorded for each fish at rates of 206 to 350 fish per hour. Only 43 fish (0.28%) died within 48 hours post-tagging. Survival of tagged (N = 122) and untagged (N = 131) groups of fish held in ponds 83 to 115 days post-tagging was >90% and near equal, except for 1 tagged and 1 untagged group where a columnaris disease outbreak occurred; survival in those groups was 68.0% and 69.1%, respectively. All tags were retained in the fish and functioned properly after 83–85 days in ponds. After 1 group of fish (N = 29) had been in a pond for 225 days, tag responses were found for all but 1 fish. The techniques used for tag placement and data recording utilized equipment generally available at most fisheries laboratories.

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Recognition of individuals or groups of fish within a population by some type of tag or body mark has been an ongoing concern of fisheries workers in management, research, and culture (Wydoski and Emery 1983, Hilborn et al. 1990). Few tagging or marking methods are available for long-term individual fish identification. Work with a passive integrated transponder (PIT) tag system in salmonids for release in the wild (Prentice et al. 1985, 1990*a*, 1990*b*), and largemouth bass (*Micropterus salmoides*) (Harvey and Campbell 1989), striped bass (*Morone saxatilis*), and red drum (*Sciaenops ocellatus*) (Jenkins and Smith 1990) brood fish has demonstrated long-term tag operation (manufacturers estimate tag life >50 years), unique individual tag codes, high survival of tagged fishes, and >90% tag retention.

The danger of fish injury during PIT tagging decreases as a technique for tag placement for a given species is developed and as fish increase in size. For example, after a technique was established, juvenile salmon 55 to 120 mm fork length were PIT tagged with survival ≥96.5% (Prentice et al. 1985, 1990*a*). Similarly, largemouth bass brood fish were PIT tagged with 100% survival while juveniles approximately 254 mm total length (TL) had 96% tagging survival (Harvey and Campbell 1989).

Initiating evaluation of aquatic vegetation control in Texana Reservoir, Jackson County, Texas, required tagging 15,300 triploid grass carp before stocking. Tagging was to aid in 2 main concerns: positive identification of the origin of grass carp emigrating from the reservoir and reduction of sample sizes, during the course of study, made possible by individual-fish data. PIT tags were chosen as the type tag to use. However, previous research had not developed a technique for optimum PIT tag placement in this species. Additionally, tagging needed to proceed at a rapid rate so as not to hinder fish farm operations. This paper describes fish handling and tagging techniques used to rapidly insert PIT tags and record needed data, as well as subsequent survival and tag retention.

We wish to thank all the personnel of Keo Fish Farms, Inc., Keo, Arkansas, for their friendliness and tolerance in allowing us to use their facility when fish were tagged. Their speed and efficiency set a pace that influenced ours. We thank members of the Texas Parks and Wildlife Department Inland Fisheries Branch for manuscript review.

Methods and Materials

Tagging Procedures

Tagging of approximately 15,300 fish took place inside a fish holding house at a fish farm near Little Rock, Arkansas, during November (water temperature = 12 − 18 C). Prior to tagging, batch lots (1,000–3,000 fish in a given raceway) of certified triploid grass carp (200–280 mm TL) were crowded and held with a meshed barrier at 1 end of a holding raceway and anesthetized with approximately 80–120 mg/l MS-222 (tricaine methane sulfonate). Anesthetic was added only to the crowded end of the raceway and was supplemented as needed to keep fish anesthetized. Air stones and/or agitators maintained oxygenation in water holding fish.

A PIT tag (Model TX 1400, BioSonics, Inc., Seattle, Wash.), approximately 10 mm long x 2 mm in diameter, was injected into the body cavity of each fish with a 12-gauge x 50-mm needle mounted on an aluminum retractable implanter (Model AY0010, BioSonics, Inc.) with the spring for automatic injector rod retraction removed. Each tag was injected 2–3 mm posterior to the left pelvic fin base at a 40–50° anterior angle to the frontal plane; penetration depth of the needle tip was near 15 mm (just inside the body wall musculature). Anterior angle was used to allow penetration between the large scales. Angle of penetration to the saggital plane was approximately 20° for the first 1,000 fish, but parallel thereafter. The bevel of the needle faced away from the body.

To keep pace with fish farm activities, tag injection, measurement and recording of TL (mm) and weight (g), and reading and recording of a 10-digit alpha-numeric unique tag code had to be completed for approximately 250 fish per hour. After injection, tags were read with a hand-held portable detector-decoder (READER; Model HS 5101, BioSonics, Inc.) to verify each tag as functional. Lack of computer facilities and the necessity to keep TL, weight, and tag code together as a unique data set for each fish precluded use of the code data storage and computer down-load capability of the READER. Timed trials before the actual tagging operation indicated a 4-person team working at 2 adjacent portable tables (1 for tagging; the other for data collecting) was required for greatest efficiency. Person 1 maintained fish in anesthesia, provided sedated fish to person 2 as needed (10–20 fish at a time placed in a water-filled, 75-liter ice chest set at waist height), and reloaded each of 5 tag implanters after they were used. Person 2 inserted tags and placed each fish after tagging in another water-filled ice chest between he and person 3. Person 3 and person 4 each wore lapel microphones plugged into the same portable audio cassette tape recorder. A second tape recorder with a general microphone was recording simultaneously as backup data storage. Person 3 removed fish from the second ice chest, measured TL and weight (using an electronic self-taring digital platform scale), and spoke the data into the tape recorders. Person 4 picked up the fish, read the tag with the READER, spoke the tag code into the tape recorders, before person 3 began with the next fish, and released the fish into an adjacent raceway. Letters in the alpha-numeric tag code were recorded by code word (A = Able, B = Baker, . . .) to insure understanding when data tapes were played back. Because cassette tapes did not record for more than 45 minutes on each side, person 4 halted data collection and changed tapes in both recorders as needed. A timer with a loud bell was set when each tape was started to sound after 35–40 minutes. All electric instruments were given constant power by operating them with AC power converters to avoid low-battery failure. Care was taken to keep electrical equipment dry by placing all instruments in protected areas or on pedestals above wet work surfaces. Plug connections were also wrapped with plastic electrician's tape to aid in moisture resistance. Needles and implanters were disassembled, maintained, and cleaned with detergent at the end of each day.

Survival and Tag Retention

Initial mortalities due to tagging were noted each morning on days after tagging and were easily determined because fish tagged on a given day went into a particular raceway for 2–5 days until loaded for delivery to the reservoir. Each morning, all dead or moribund fish were removed from each raceway and necropsied to determine cause of death and that the tag was retained.

Long-term survival and tag retention were measured by tagging 3 groups of fish (N = 122), using the same procedures as above, and stocking those and 3 untagged (control) groups of fish (N = 131) in separate, similar hatchery ponds (0.20–0.29 ha), each with similar aquatic vegetation, near Ingram, Kerr County, Texas. Ponds were drained, fish recovered, and tags read after 83 to 115 days. One

tagged group was returned to a pond and held for an additional 141 days (225 total days). Surviving fish were needed for other projects and therefore could not be sacrificed to observe internal condition of fish and tags. However, a columnaris disease outbreak occurred in 1 tagged and 1 untagged group which allowed necropsy of 7 fish that died during the disease. Necropsy was done to determine tag location and condition of tissue near each tag.

Differences in growth were analyzed between the 2 tagged and 2 untagged groups which had not experienced the columnaris disease. Data for the 2 tagged groups were pooled and TL increments from pond stocking to draining were determined by differences in the TL for each fish due to PIT-tag identification and by mean differences for the groups combined. Data for the 2 untagged groups were pooled and TL increments from pond stocking to draining were determined only by mean differences for the groups combined. Analysis for differences in TL increments between tagged and untagged fish was done by *t*-test between means of independent samples (Snedecor and Cochran 1967).

Results

Tagging Procedures

The rate of tagging was satisfactory to keep pace with fish farm activities during the first tagging-operation day (276 fish tagged per hour, Table 1). However, as experience was gained, tagging rate was increased to >300 fish per hour. The most time-consuming portion of the tagging operation was recording the TL, weight, and tag-code data set for each fish. All electronic equipment, except the platform scales, functioned well throughout the 7-day operation. The platform scales would occasionally get wet and malfunction, thereby requiring a replacement scale.

Initial Survival and Tag Retention

Only 43 of the 15,344 fish tagged (0.28%) died within 48 hours after tagging (Table 1). Mortalities usually occurred within 24 hours post-tagging (Table 1); no

Table 1. Numbers of grass carp (200–280 mm TL) PIT tagged each day and initial post-tagging mortalities, winter 1989.

Tagging operation day	*N* tagged		Post-tagging mortality	
	Total	Per Hour	24-hour	48-hour
1	2,070	276	21	4
2	2,005	267	5	2
3	2,408	301	5	0
4	2,797	350	1	0
5	2,613	327	2	0
6	2,625	328	2	0
7	826	206	1	0
Total	15,344		37	6

mortalities were observed after 48 hours post-tagging. The highest tagging mortality occurred during the first tagging-operation day (Table 1) and probably involved the first 1,000 fish tagged. Necropsies revealed that of the 43 mortalities, 18 were due to punctured gall bladders, 3 to punctured livers, and 2 to punctured air bladders, while 20 deaths could not be attributed to a particular injury. All of the dead fish had retained their tags.

Long-term Survival and Tag Retention

Survival of tagged and untagged groups of fish after 83 to 115 days in ponds was >90% and near equal in all but groups A and D (Table 2). Groups A and D each experienced a columnaris disease outbreak; their survival was 68.0% and 69.1%, respectively. Survival was 90.6% for group A fish which survived columnaris and were restocked for an additional 141 days (Table 2).

No difference in growth (TL) in ponds was found when individual tagged-fish increments ($P < 0.05$) or mean tagged-fish increments ($P < 0.05$) were compared to mean untagged-fish increments.

All tags remained in the fish and functioned properly at the end of 83–85 days. After group A had been restocked and retrieved a second time (225 total days in pond), tag response was found for all but 1 fish (Table 2). Necropsy of 7 group A fish, which died during the columnaris outbreak, revealed tags were encapsulated in mesentery tissue and properly located just anterior to the pelvic girdle next to the body wall. No signs of inflammation or irritation were observed in internal tissues around these tags. No tagged fish recovered from ponds at any time showed any external evidence of tissue damage or infection at the tag injection site.

Table 2. Summary of growth, survival, and tag loss of PIT-tagged and untagged (control) grass carp held in separate (by group) 0.20-to 0.29-ha hatchery ponds near Ingram, Texas, during spring and summer 1990.

	At pond stocking		At pond draining				
Group	*N* fish	Mean total length mm (SD)	*N* fish	Mean total length mm (SD)	Days in pond	% survival	Tag loss[a]
Tagged fish							
A	50	287(17.4)	34	308(22.5)	84	68.0[b]	0
A[c]	32	308(22.5)	29	485(17.9)	141	90.6	1
B	20	347(16.1)	19	482(15.0)	85	95.0	0
C	20	346(16.2)	19	489(29.4)	83	95.0	0
Untagged fish							
D	97	299(19.9)	67	357(11.6)	115	69.1[b]	—
E	13	348(15.3)	13	476(11.3)	85	100.0	—
F	21	357(10.6)	19	506(10.0)	83	90.5	—

[a]Because fish could not be sacrificed, tag loss was lack of tag code recognition by the READER.
[b]Groups A and D each experienced an outbreak of columnaris disease.
[c]After the columnaris disease, surviving fish of group A were returned to a pond for additional time.

Discussion

High survival of PIT-tagged triploid grass carp and high tag retention and function rates over time after tag placement were similar to those in studies done with other fishes (Harvey and Campbell 1989, Prentice et al. 1990*a*, Jenkins and Smith 1990) and indicate PIT tags offer a dependable tool for individually marking fish. Also, PIT tags apparently had little or no effect on fish growth, similar to observations made for salmonids (Prentice et al. 1985, 1990*a*). The columnaris outbreak, although not desirable at the time, was useful in that it provided an opportunity to directly compare survival between tagged and untagged fish under a situation of increased physiological stress. Similarly in survival of the 2 groups of fish with columnaris strengthens the conclusion that PIT tags are not themselves stressful to the host fish.

Tag response was not found for 1 of 29 fish (3.4%) after 225 total days in a pond. Because fish could not be sacrificed, the lack of tag function could have been due to the tag having left the fish's body or a present but non-functional tag.

The greatest concern during tagging was prevention of injury to internal organs, which was greatly reduced in this study with the anterior penetration angle and change of sagittal angle to zero degrees. However, another approach to prevent internal organ injury in large (>250 mm TL) grass carp might be intramuscular tag placement as described by Jenkins and Smith (1990). Consistent tag placement was felt to be important in this study to minimize internal injury, similar to the findings of Prentice et al. (1985). Use of the pelvic fin as a quick body reference for penetration point was beneficial for consistent tag placement.

We were able to complete tagging and data collection at a rapid rate equivalent to that described by Prentice et al. (1990*b*) who used a semiautomated tag injector and computer-interfaced data recording system. Although their system would probably have increased our speed, we did not have the computer-related hardware. Except for the PIT tag implanter and READER, equipment we used is generally already present at most fisheries laboratories, easily set up and used in the field, and easily set up in redundant fashion to facilitate equipment backup in case of component failure. Electronic platform scales might be placed inside a thin plastic bag to prevent them from becoming wet, if the self-taring feature is not hampered.

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