Effects of different tagging protocols on survival, growth, and tag retention in juvenile least cisco
Coregonus sardinella

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Abstract

Passive integrated transponder (PIT) and T-bar anchor tags are often used in fisheries evaluations. To collect unbiased data, tags must not alter behavior, growth, or survival and have high retention rates. We examined tag retention, growth, and survival of age-2 juvenile least cisco *Coregonus sardinella* over a 60-day experiment following handling, anesthesia, tagging, and recovery using PIT and T-bar anchor tags. Fish were assigned to one of 12 treatment groups (20 fish per group), with individuals first assigned to one of three tagging treatments (internally implanted PIT tag, externally attached T-bar anchor tag, no tag [control]). Tag treatment groups were next assigned to a pre-handling anesthesia treatment (clove oil or no anesthetic) and a post-handling recovery treatment (elevated [10 ppt] saline or ambient [3 ppt] saline solution). Tag retention for least cisco was greater for fish that received PIT tags (100%) compared to T-bar anchor tags (25–45%). The majority of T-bar anchor tag loss (50%) occurred between days 25 and 45 of the experiment. Fish in all treatments exhibited positive growth, with no differences among treatments for specific growth rates, Fulton condition, or survival (80–100%). Most mortality (13 of 18 fish) occurred by day 5 of the study. The lowest survival (80%) occurred for fish that were not anesthetized, received PIT tags, and recovered in a 10-ppt saline solution. Our results indicate that T-bar anchor tags are sufficient for short-term studies (<30 d) involving least cisco, but PIT tags are preferred for longer evaluations (>30 d) of this species.

1. Introduction

Tagging fish has been widely used in fisheries management to examine behavior, migration, growth, survival, and recruitment (Taylor and Arndt, 2013; Smith et al., 2015). For field applications, two basic assumptions must be met for the collection of unbiased data: (1) tag loss must be minimal or at least known; and (2) tags cannot alter fish behavior, growth, or survival (Pine et al., 2012). However, these assumptions may not be met because tag retention is often dependent on fish species, tag type, attachment location, and body size (Buckmeier and Reeves, 2012). Further, alterations to growth and survival due to tagging-induced stress may be compounded for sensitive species (Skalski et al., 2009). As a result, species-specific evaluations are necessary to identify proper tag selection, application, and handling techniques.

Externally attached and internally implanted tags are often used to identify individual fish in tagging studies (Rude et al., 2011; Taylor and Arndt, 2013). In selecting a tagging method, factors such as cost, tag retention, impacts on study species, and study duration must be addressed (Buzby and Deegan, 1999). Tag selection for smaller species or life stages is further complicated because small individuals typically have lower tag-related survival (Skalski et al., 2009). To reduce mortality, anesthesia may be necessary to lessen the effects of stress and injury (Ross and Ross, 2008). Previous research has also shown that recovery by adding salt to a recovery bin can further reduce stress and mortality for anesthetized fish (Carneiro and Urbinati, 2002).

For this study, we examined tagging effects on least cisco *Coregonus sardinella*, an important subsistence and recreational resource throughout the Beaufort, Chukchi, and Bering Sea drainages (Reist and Bond, 1988). Because least cisco do not respond well to tagging and handling (Dupuis 2010), there is a need to conduct controlled experiments to quantify tag loss

and tagging-induced stress on growth and survival to identify appropriate handling and tagging protocols. This study examined tag retention, growth, and survival of juvenile least cisco following handling, anesthesia, tagging, and recovery using internal and external tags to identify the least invasive and stressful procedures for tagging juvenile coregonids.

2. **Methods**

2.1. *Experimental design*

Least cisco were cultured from eggs fertilized from spawning adults collected in the Tanana River, Alaska, following established protocols (T. Sutton, unpubl. data). Five days prior to the start of the experiment, five least cisco were stocked into each of 48 aquaria (240 fish total) to allow acclimation to experimental conditions. Tag treatments included internal implantation of a passive integrated transponder (PIT) tag, external attachment of a T-bar anchor tag, and no tag (control group), with 16 tanks assigned to each tag treatment. Anesthesia and recovery was also evaluated for tag treatments. Half the tanks in each tag group were assigned the anesthesia treatment (anesthesia prior to handling and tagging), while the other half of the tanks were assigned to the non-anesthesia treatment (no anesthesia prior to handling and tagging). Within each treatment, half the tanks were assigned to an elevated saline solution treatment (anesthesia recovery in a 10-ppt saline solution) and the other half of the tanks were assigned to a non-elevated saline solution treatment (anesthesia recovery in ambient freshwater [3 ppt]). Thus, a 3 x 2 x 2 (tag x anesthesia x recovery treatment) factorial design was conducted, with each treatment having four replicates and individual aquaria being the sampling unit.

2.2. *Fish tagging*

At the start of the experiment, least cisco in the anesthesia treatment were placed in a 20 mg/L clove oil solution (an approved anesthetic in Alaska) until they could not maintain equilibrium (Prince and Powell, 2000). Fish were measured for total length (TL; 1 mm) and wet weight (WW; 0.01 g), tagged (if in a tagging group), and placed into an aerated tub containing their assigned recovery solution. Similarly, fish not anesthetized were measured for TL and WW, tagged (if in a tagging group), and placed into their assigned recovery solution. Regardless of tag and anesthesia treatment, fish remained in their assigned recovery solution until they could maintain equilibrium before being restocked into their assigned experimental aquarium.

Tagging procedures for least cisco followed standard protocols for the implantation and attachment of PIT and T-bar anchor tags, respectively (Rude et al., 2011; Bodine and Fleming, 2014). Fish in the internal implant tag group had a PIT tag (Biomark Model HPT8, 134.2 kHz, 8.4 x 1.4 mm, 0.02 g in air; Biomark, Boise, Idaho) injected into their peritoneal cavity just anterior to the pelvic fins and immediately to the right of the midventral line parallel with the long axis of the body. A Biomark syringe implanter (MK165) and a 16-gauge needle (N165 needle; 6-mm insertion depth, 1.5-mm needle width) was used to implant the PIT tag. For fish in the external attachment tag group, a T-bar anchor tag (Floy Model FD-68B FF, 34 x 1 mm, 0.10 g in air; Floy Tag and Manufacturing, Inc., Seattle, Washington) was injected below the left side of the dorsal fin between the pterygiophore bones using a Floy pistol-grip implanter and a fine fabric needle (6-mm insertion depth, 1-mm needle width). During tagging, fish were immobilized in a wetted cradle and the tag number was recorded using a Biomark 601 handheld reader (PIT tags) or visual detection (T-bar anchor tags). All equipment was sanitized in a 100% povidone-iodine solution and rinsed with distilled water before tagging each fish.

2.3. *Experimental protocols*

During the 60-d experiment, fish were fed daily a 4% body weight ration of formulated finfish starter feed. Each day, uneaten food and fecal material was removed and each aquarium was examined for dead fish and shed tags. For dead fish, the following information was recorded: tag number (if in a tag treatment group), TL, WW, and date of mortality. At the end of the experiment, all fish were euthanized, measured for TL and WW, and their tag recorded.

2.4. *Data analyses*

Tag retention (TR), specific growth rate (SGR), final TL and WW, Fulton condition factor (K), and survival rate (SR) were evaluated for least cisco. Mean TR was calculated as the ratio of the number of fish that retained their tag to the number of fish tagged x 100 and specific growth rate (%TL and %WW per day) was calculated as $SGR_{TL} = (\log_e [\text{final TL}] - \log_e [\text{initial TL}]) / \text{experiment days} \times 100$ and $SGR_{WW} = (\log_e [\text{final WW}] - \log_e [\text{initial WW}]) / \text{experiment days} \times 100$. Other metrics were calculated as $K = (WW / TL^3) \times 10^4$ and $SR = (1 - N_1 / N_0) \times 100$, where N_1 and N_0 were the number of fish at the end and onset of the experiment, respectively. To differentiate growth criteria among the treatment combinations, data were analyzed with a factorial multivariate analysis of variance (MANOVA), which was selected because of correlated response variables. Tag retention among treatments were evaluated by comparing Kaplan-Meier product limit survival curves with a Log-Rank test, and pairwise multiple comparisons were conducted using a Holm-Sidak test.

3. Results

3.1. *Tag retention*

Tag retention by least cisco was significantly greater for fish with PIT tags than for individuals with T-bar anchor tags ($\chi^2 = 84.54$, $P < 0.001$). Pairwise comparisons revealed that

tag type was the only factor that produced significant differences in tag retention ($F = \text{range [13.03-29.81]}$, $P = \text{range [<0.001-0.003]}$). No PIT tags were lost during the experiment (100% retention). In contrast, tag retention for fish with T-bar anchor tags ranged from 25-45%. Tag loss patterns were similar among anesthesia and recovery solution treatments, with most T-bar anchor tags lost between experiment days 25 and 45 (Fig. 1).

3.1. *Body size and growth*

At the beginning of the experiment, there were no differences in TL (mean = 150 mm; range = 136-158 mm), WW (mean = 26.6 g; range = 18.3-31.6 g), or condition (mean = 1.08; range = 0.83-1.65) of least cisco among treatments (TL: $F = 0.88$, $P = 0.56$; WW: $F = 0.86$, $P = 0.59$; K: $F = 0.92$, $P = 0.53$). Fish in all treatment groups exhibited increases in growth over the experiment, with control fish exhibiting slightly higher growth rates and attaining larger body sizes than tagged fish (Fig. 2). Final mean and specific growth rates, regardless of treatment, did not differ among treatments ($F = \text{range [0.62-1.543]}$, $P = \text{range [0.14-0.71]}$; Fig. 2).

3.2. *Survival*

Survival of least ciscoes was not affected by tagging treatments ($\chi^2 = 11.03$, $P = 0.44$). The lowest survival rate (80%) was for fish with PIT tags that were not anesthetized using clove oil and were allowed to recover in a 10-ppt saline solution. Survival rates ranged from 90-100% for the other 11 treatment groups. Necropsy examinations of fish that died during the experiment indicated that mortalities were not due to ruptured internal organs, hemorrhaging at tag locations, or secondary infection. Thirteen of 18 mortalities occurred within 5 days of the experiment start, suggesting handling stress as the most likely cause of mortality.

4. Discussion

Retention of T-bar anchor and PIT tags by least cisco in our study revealed differences between tag types, which is consistent with previous studies (Rude et al., 2011; Bodine and Fleming, 2014). While no PIT tags were shed by least cisco, retention of T-bar anchor tags was high until day 30 (76%). Smallmouth bass *Micropterus dolomieu* (156–376 mm TL) also had high retention of T-bar anchor tags (76%) for the first 1.5 months, but retention declined to 48% over the next 2.5 months (Walsh and Winkelman, 2002). Juvenile Chinook salmon *Oncorhynchus tshawytscha* implanted with PIT tags showed a 99.996% retention rate over a 28-day period, with overall retention rates for juvenile Pacific salmon *Oncorhynchus* spp. greater than 99% (Dare, 2003). Our results corroborate previous research showing that fish tagged with PIT tags generally have a higher retention rate than individuals with T-bar anchor tags (Rude et al., 2011).

Current Alaska Department of Fish and Game tagging procedures do not require anesthetizing fish unless tags are implanted surgically. As a result, fish are not anesthetized for length and weight measurements, removal of scales, or when tagged with T-bar anchor or PIT tags. Our results support the current tagging protocol of not anesthetizing least cisco. However, most of the fish mortalities in our study (73%) occurred within the first five days post-tagging, most likely due to handling- and tagging-induced stress. Without individual trials to evaluate pre- and post-stress abatement treatments independently, we were unable to identify if stress responses that lead to mortality originated prior to anesthesia. Additional remedial measures should be examined to reduce handling and tagging mortality. For example, keeping fish fully submerged during tagging, tagging fish in a darkened room, evaluating other anesthetics, and recovery in higher or lower saline solutions may reduce mortality (Weirich et al., 1992).

Regardless of tag type and anesthetic/recovery treatment, fish in this study did not exhibit differences in growth and survival. In previous studies, external tags have been shown to negatively affect growth of smaller fish (Rikardsen, 2000; Rikardsen et al., 2002). Conversely, other studies have shown that PIT tags appear to have minimal impacts on growth and survival (Ruetz et al., 2006; Wagner et al., 2007). The similarities in growth and survival rates for least cisco among PIT tag, T-bar anchor tag, and control groups may be explained by factors such as tagger experience, tag weight, and/or biotic and abiotic conditions (Cooke et al., 2010). Tagging was conducted by experienced personnel, and the minimum tag to fish weight ratio was 0.25% for PIT tags and 1.25% for T-bar anchor tags. While it is generally accepted that internal and external tags/transmitters should not exceed 2% of fish body weight (Pine et al., 2012), some studies have recommended that external tags weigh no more than 1.25% of fish body weight (Brown et al., 1999; Sutton and Benson, 2003). The conservative tag to fish weight ratio for both tag types in this study may have alleviated potential negative effects on growth and survival. Nonetheless, results from controlled experiments, while useful to resource managers, should be considered best-case scenarios.

Although not statistically different, survival rates of least cisco varied considerably within and between treatments. For instance, the lowest survival rate occurred for fish that received PIT tags, but were not anesthetized and were allowed to recover in a 10-ppt saline solution. Alternatively, four treatments had 100% survival. As a result, our experiment may have suffered from a lack of power to detect differences among survival rates. The survival rates observed in this study are similar to previous studies involving PIT and T-bar anchor tags (Wagner et al., 2007). For future studies, increasing the number of treatment replicates could aid in elucidating differences, if any, in survival from tagging and handling procedures.

Considerations such as cost, tag retention rates, impacts on study species, and study duration must also be made when choosing an appropriate tag type (Buzby and Deegan, 1999). For short-term studies (< 30 days), our results showed that T-bar anchor tags are a satisfactory tag type for least cisco. For longer studies (> 30 days), the insertion of PIT tags is preferred for least cisco. For short-duration studies (< 30 days), there is a substantial cost savings in using T-bar anchor tags instead of PIT tags. If funding is sufficient, then PIT tags are preferred due to their superior retention.

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References

- Bodine, K.A., Fleming, P., 2014.** Retention of PIT and T-bar anchor tags in blue catfish. N. Am. J. Fish. Manage. 34, 68–71.
- Brown, R.S., Cooke, S.J., Anderson, W.G., McKinley, R.S., 1999.** Evidence to challenge the “2% rule” for biotelemetry. N. Am. J. Fish. Manage. 19, 867–871.
- Buckmeier, D.L., Reeves, K.S., 2012.** Retention of passive integrated transponder, T-bar anchor, and coded wire tags in lepisosteids. N. Am. J. Fish. Manage. 32, 573–576.
- Buzby, K., Deegan, L., 1999.** Retention of anchor and passive integrated transponder tags by Arctic grayling. N. Am. J. Fish. Manage. 19, 1147–1150.
- Carneiro, P.C.F., Urbinati, E.C., 2002.** Transport stress in matrinxã, *Brycon cephalus*

(Teleostei: Characidae), at different densities. *Aq. Intl.* 10, 221–229.

Cooke, S.J., Woodley, C.M., Eppard, M.B., Brown, R.S., Nielsen, J.L., 2010. Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging studies. *Rev. Fish Biol. Fish.* 21, 127–151.

Dare, M.R., 2003. Mortality and long-term retention of passive integrated transponder tags by spring Chinook salmon. *N. Am. J. Fish. Manage.* 23, 1015–1019.

Dupuis, A.W., 2010. Reproductive biology and movement patterns of humpback whitefish and least cisco in the Minto Flats-Chatanika River complex, Alaska. Master's thesis, University of Alaska Fairbanks.

Pine, W.E., Hightower, J.E., Coggins, L.G., Lauretta, M.V., Pollock, K.H., 2012. Design and analysis of tagging studies. In: Zale, A.V., Parrish, D.L., Sutton, T.M. (Eds.), *Fisheries Techniques*, 3rd edition. American Fisheries Society, Bethesda, MD, pp. 521–572.

Prince, A., Powell, C., 2000. Clove oil as an anesthetic for invasive field procedures on adult rainbow trout. *N. Am. J. Fish. Manage.* 20, 1029–1032.

Rikardsen, A.H., 2000. Effects of floy and soft VI alpha tags on growth and survival of Arctic charr. *N. Am. J. Fish. Manage.* 20, 719–728.

Rikardsen, A.H., Woodgate, M., Thompson, D.A., 2002. A comparison of floy and soft VI alpha tags on hatchery Arctic charr, with emphasis on tag retention, growth, and survival. *Env. Biol. Fish.* 64, 269–273.

Reist, J.D., Bond, W.A., 1988. Life history characteristics of migratory coregonids of the lower Mackenzie River, Northwest Territories, Canada. *Fin. Fish. Res.* 9, 133–144.

Ross, L.G., Ross, B., 2008. *Anesthetic and Sedative Techniques for Aquatic Animals*, 3rd

edition. Blackwell Scientific Publications, Oxford, UK.

- Rude, N.P., Whitley, G.W., Phelps, Q.E., Hirst, S., 2011.** Long-term PIT and T-bar anchor tag retention rates in adult muskellunge. *N. Am. J. Fish. Manage.* 31, 515–519.
- Ruetz III, C.R., Earl, B.M., Kohler, S.L., 2006.** Evaluating passive integrated transponder tags for marking mottled sculpins: effects on growth and mortality. *Trans. Am. Fish. Soc.* 135, 1456–1461.
- Skalski, J.G., Buchanan, R.A., Griswold, J., 2009.** Review of marking methods and release-recapture designs for estimating the survival of very small fish: examples from the assessment of salmonid fry survival. *Rev. Fish. Sci.* 17, 391–401.
- Smith, N.J., Sutton, T.M., Savereide, J., 2015.** Seasonal movement patterns of Inconnu in an Arctic estuary delta complex. *N. Am. J. Fish. Manage.* 35, 698–707.
- Sutton, T.M., Benson, A.C., 2003.** Influence of external radio transmitter shape and size on tag retention and growth of juvenile lake sturgeon. *Trans. Am. Fish. Soc.* 132, 1257–1263.
- Taylor, J.L., Arndt, K.A., 2013.** Variability in burbot cohort abundance at juvenile and adult stages in Columbia Lake, British Columbia. *Trans. Am. Fish. Soc.* 142, 1705–1715.
- Wagner, C.P., Jennings, M.J., Kampa, J.M., Wahl, D.H., 2007.** Survival, growth, and tag retention of age-0 muskellunge implanted with passive integrated transponders. *N. Am. J. Fish. Manage.* 27, 873–877.
- Walsh, M.G., Winkelman, D.L., 2002.** Short-term retention of floy anchor tags by stream-dwelling smallmouth bass. *Proc. Ann. Conf. SE Assoc. Fish Wildl. Agen.* 56, 38–43.
- Weirich, C.R., Tomasso, J.R., Smith, T.I.J., 1992.** Confinement and transport-induced stress in white bass *Morone chrysops* x striped bass *M. saxatilis* hybrids: effect of calcium and salinity. *J. World Aq. Soc.* 23, 49–57.

Fig. 1. Retention (%) of T-bar anchor tags for juvenile least ciscoes over the experiment by anesthesia and recovery treatment.

Fig. 2. Juvenile least cisco growth, body size, and condition ($\pm$ SD) among treatment combinations at the end of the laboratory experiment. The letter combinations on the x axis refer to the treatment, with the first letter being anesthesia (clove oil [C] or no anesthesia [N]), second

being tag type (PIT [P], T-bar anchor [T], or no tag [N], and the third being recovery (freshwater [F] or saline [S]) treatment. Values are the raw data from four replicates per treatment.