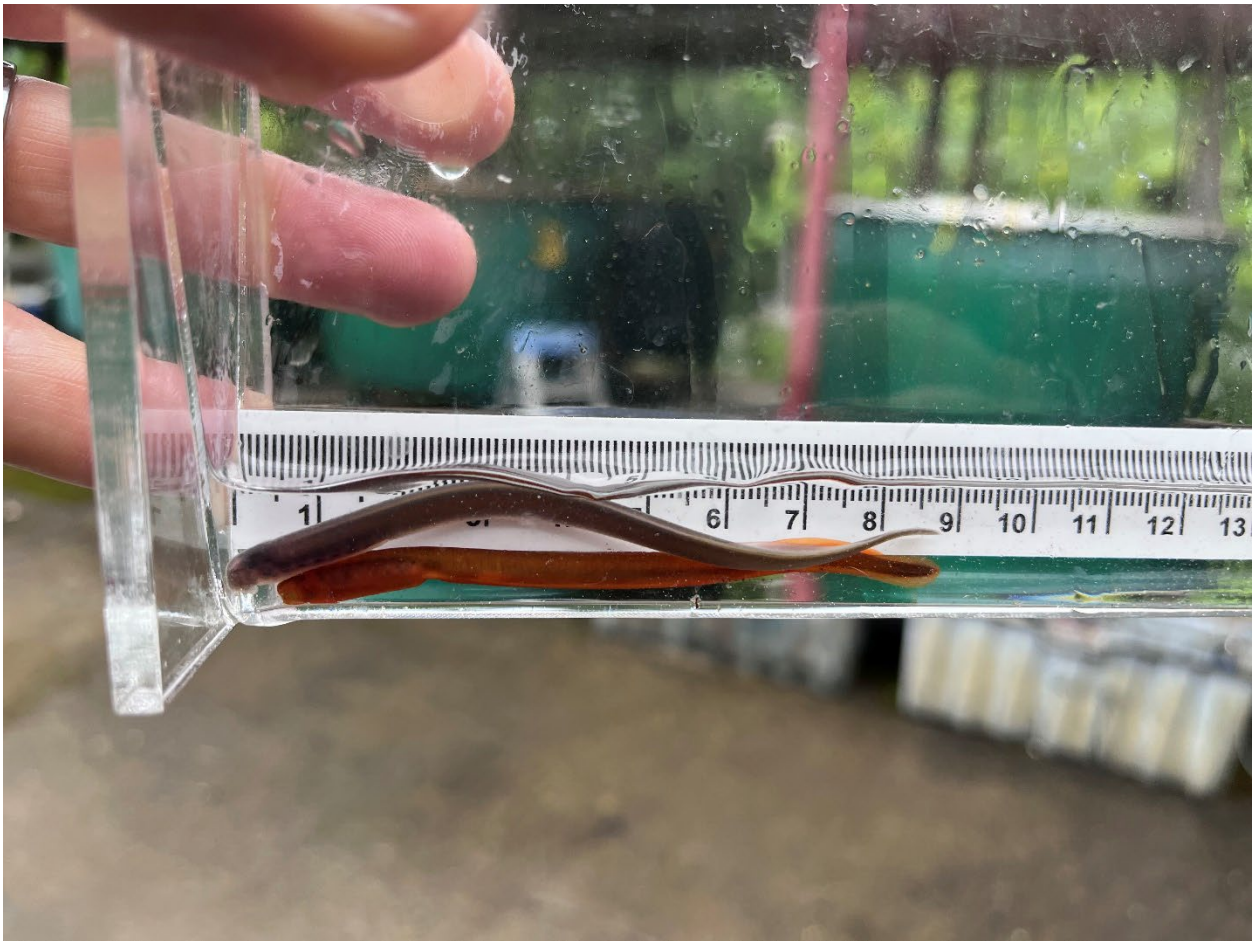
U.S. Fish and Wildlife Service

Columbia River Fish & Wildlife Conservation Office

An evaluation of batch marking techniques for larval lampreys

2024



Kayla L. Kelley, Timothy J. Blubaugh, William G. Simpson, and Julianne E. Harris
U.S. Fish and Wildlife Service
Columbia River Fish & Wildlife Conservation Office
Vancouver, WA 98683

On the cover:
Experimental (bottom) and control (top) fish after the Bismarck Brown dye experiment.
Photo Credit: Timothy Blubaugh (USFWS)

Disclaimers:
The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the federal government.

The correct citation for this report is:
Kelley, K.L., Blubaugh, T.J., Simpson, W.G., and Harris, J.E. (2024). An evaluation of batch marking techniques for larval lampreys. U.S. Fish & Wildlife Service, Columbia River Fish & Wildlife Conservation Office, Vancouver, Washington (USA)

An evaluation of batch marking techniques for larval lampreys

Work funded by

U.S. Fish and Wildlife Service

and authored by

Kayla L. Kelley 1
Timothy J. Blubaugh 2
William G. Simpson 3
Julianne E. Harris 4

U.S. Fish and Wildlife Service
Columbia River Fish & Wildlife Conservation Office

1211 SE Cardinal Court, Suite 100
Vancouver, WA 98683

Pacific Lamprey *Entosphenus tridentatus* is an ecologically important anadromous species native to the Pacific Northwest region and a species of concern in the Columbia River basin (Close et al., 2002, Wang and Schaller 2015). Pacific Lamprey have declined in distribution due to anthropogenic impacts such as dams, climate change, and other habitat alterations (Clemens et al. 2017, Wang et al. 2020, Hess et al. 2021). As research and conservation efforts increase for Pacific Lamprey and other lampreys (including *Lampetra spp.*), marking and tagging techniques should be further evaluated for use in future studies and monitoring. Common tagging methods previously evaluated for larval lamprey include coded wire tags (CWT), visible implant elastomer tags (VIE), and passive integrated transponder tags (PIT) (e.g., Stone et al. 2006, Meeuwig et al. 2007, Silver et al. 2009, Hanson and Barron 2017, Moser et al. 2017). However, these tagging methods have limitations, such as CWTs require euthanization to retrieve the tag code, VIE tags have reduced detectability after metamorphosis, and all likely have minimum body size thresholds for tag implantation (Hanson and Barron 2017, Meeuwig et al. 2007). Many of these techniques also require fish anesthetization (CWT, VIE, PIT). In our study, we investigated several simple batch marking techniques to assess mortality and effectiveness for larval lampreys.

Our study was conducted at the USGS Western Fisheries Research Center in Cook, WA USA, June 9-10, 2022. All larval lampreys (n = 48, TL 64 – 141 mm) were provided under permitted research. We evaluated three batch marking techniques previously used for other fish species: 1) immersion staining with Bismarck Brown; 2) immersion staining with fluorescein; and 3) subcutaneously applied photonic ink mark applied using a jet injector (BMX2000 MICRO-Ject, NewWest Technologies, Inc.; Santa Rosa, CA). Larval lampreys were categorized based on their total length, with fish < 90mm defined as medium and fish ≥ 90mm defined as large. Each experiment consisted of 10 experimental fish and two control fish. Fish were marked on Day 1 (June 9) and mortality, burrowing behavior, and mark visibility were assessed on Day 2 (June 10).

Bismarck Brown - Bismarck Brown dyeing techniques have been examined for many fish species (Deacon 1961, Ward and Verhoeven 1963, Ewing 1990, Gaines and Martin 2004, Briand et al. 2005, Garner et al. 2019) and used for juvenile *Lampetra ayresii* by Beamish and Youson (1987). Solution dilutions reported in these studies ranged from 1:5,000 to 1:400,000 with immersion durations from 30 minutes to 6 hours. At higher concentrations Bismarck Brown was lethal to age-0 salmonids during immersion (Ward and Verhoeven 1963) and up to 3 weeks post-immersion (Ewing 1990), but appears safe for other, non-salmonid, species (Deacon 1961). Longer immersion times have a greater effect on mark retention although durations of 6 hours can also lead to higher mortality rates. In our study, we used two dilutions of Bismarck Brown Y (50% dye content), 1:15,000 and 1:7,500, with immersion durations of 1 hour. We increased our concentrations relative to what is commonly used for juvenile salmon due to the limited exposure time and the darker pigmentation of larval lamprey.

Immersion solutions contained 0.25g (1:15,000) and 0.5g (1:7,500) of Bismarck Brown in 3.75L of water. Dilutions were mixed prior to fish being added to the solution and an aerator was placed in the bucket to provide oxygen for the duration of dyeing. Fish were

measured without anesthetic and a proportional number of medium and large larvae were placed in each dye bucket. After 1 hour, fish were removed from the dye buckets and placed in buckets with fresh water and a burrowing substrate consisting of approximately 5 cm of sand. A net covering was placed over the top of the bucket to prevent fish from escaping. All control fish were placed in a separate bucket. All three buckets were then placed in a large holding container filled with circulating water and were held overnight. Mortalities, mark visibility, and burrowing behavior were recorded on Day 2. Fish were retrieved individually from experimental and control group buckets in a random order by one researcher and placed in front of another researcher to prevent mark visibility bias in comparison to control fish. Experimental and control fish were anesthetized with MS-222 for ease of handling while investigating marking effectiveness.

During the hour dyeing process, biologists noted that some larval lampreys were laying on the bottom, mostly motionless, and others were poking their heads out of the water. On Day 2, Bismarck Brown marks were visible on all (100%) experimental larval lampreys at both dilution levels with 95% Binomial Confidence Intervals (CI) of 69% - 100%. However, both dilutions resulted in high mortality. Mortality for the 1:15,000 dilution was 70% (CI: 35% - 93%) and mortality for the 1:7,500 dilution was 100% (CI: 69% - 100%). All deceased fish were not burrowed, whereas all surviving fish had burrowed. Previous studies using similar immersion times and more dilute solutions found that Bismarck Brown caused only minimal mortality for multiple fish species (Gaines and Martin 2004, Briand et al. 2005, Garner et al. 2019). High dye dilution concentrations used in this study may have led to increased mortality. Considering the high visibility of the stain we observed, further experimentation with varying dilutions and duration times are recommended to investigate Bismarck Brown as a batch marking method for larval lamprey.

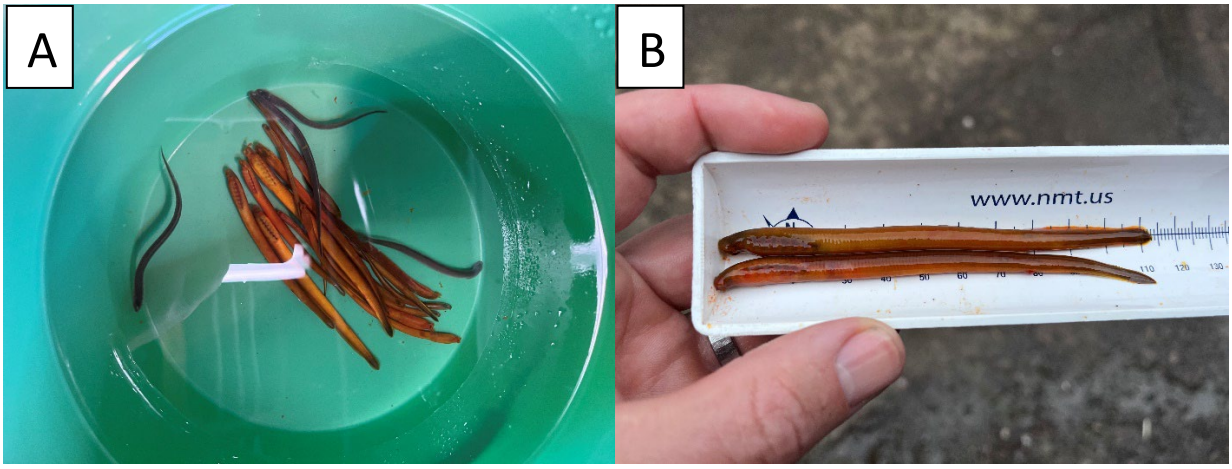


Figure 1. (A) Experimental and control larval lamprey on Day 2 after being inspected for visibility of Bismarck Brown as a batch marking technique. (B) Two larval lamprey experimentally marked with Bismarck Brown on Day 2. Credit: Timothy Blubaugh (USFWS).

Fluorescein - Fluorescein, a bright yellow-green dye that fluoresces under the proper wavelength of light, has been extensively studied as a method for marking fish. Fluorescein marks otoliths, as well as fin rays, scales, and other calcified tissue (Hill and Quesada 2010, Gilbert et al. 2020). A proprietary fluorescein detector is commonly used to examine dyed fish with mark visibility improved by viewing in darkness. Additionally, environmental factors and exposure to the sun reduce mark retention (Hill and Quesada 2010). We did not use specialized equipment because the purpose of our study was to assess mark visibility in the field using a readily available handheld black light already utilized for other marking methods (e.g., VIE).

Because lamprey do not have scales and calcified tissue like many other fishes, we followed marking methods from a study that investigated fluorescein marking of lesions and ulcerations on fish skin (Noga and Udomkusonsri, 2002). We used 2.0g of fluorescein in 4.0L of water. Similar to our Bismarck Brown study, dilutions were mixed prior to fish being added to the solution and an aerator was placed in the bucket to provide oxygen for the duration of dyeing. Fish were measured without anesthetic and a proportional number of medium and large categorized individuals were placed in the dye bucket. After the 12 minutes of immersion, fish were removed from the dye buckets and placed in buckets with fresh water and a burrowing substrate consisting of approximately 5 cm of sand. A net covering was placed over the top of the bucket to prevent fish from escaping. All control fish were placed in a separate bucket. Both buckets were then placed in a large holding container filled with circulating water and held overnight. As done with Bismarck Brown marked fish, mortalities, mark visibility, and burrowing behavior were recorded the following day (Day 2). Fish were retrieved individually from experimental and control group buckets in a random order by one researcher and placed in front of another researcher to prevent mark visibility bias in comparison to control fish. Experimental and control fish were anesthetized with MS-222 for ease of handling while a black light was used in a dimly lit room to increase visibility of the fluorescent mark.

No mortalities (0%, CI: 0% - 31%) were observed for larval lampreys marked with fluorescein and all fish were highly active immediately after dyeing and on Day 2. On Day 2, all fish were burrowed (100%, CI: 69% -100%); however, no mark was visible on any fish (0%, CI: 0% - 31%). The absence of noticeable marks may be due to lamprey having minimal calcified tissue and lacking anatomical features to which fluorescein could bind (e.g., fin rays, scales, skin damage).

Figure 2. Larval lamprey immersed in the fluorescein dye during experimentation. Photo credit: William Simpson (USFWS).

Jet injection – Studies marking small and juvenile fishes with jet injectors and jet inoculators (Panjet, Dermojet) have been conducted (Moffet et al. 1997, Dietrich and Cunjak 2006, Pitsch et al. 2021). Moffet et al. (1997) found a significant decrease in survival with this marking method compared to control fish. However, jet injected marks can be retained for long periods of time (Moffet et al. 1997, Pitsch et al. 2021).

A fluorescent-colored ink formula was applied to the caudal fin using a high-pressure jet injector. Larval lampreys were not anesthetized while administering the mark to evaluate effectiveness when research needs preclude the use of anesthetic. Each lamprey was marked outdoors by one researcher holding the individual on a board while a second researcher operated the jet injector. Fish were measured and a proportional number of medium and large categorized individuals were marked using the jet injector. Once injected, fish were placed in buckets with fresh water and a burrowing substrate consisting of approximately 5 cm of sand. A net covering was placed over the top of the bucket to prevent fish from escaping. All control fish were placed in a separate bucket. Both buckets were then placed in a large holding container filled with circulating water and held overnight. Fish mortalities, mark visibility, and burrowing behavior were recorded the following on Day 2. Fish were retrieved individually from experimental and control group buckets in a random order by one researcher and placed in front of another researcher to prevent mark visibility bias in comparison to control fish. Experimental and control fish were anesthetized with MS-222 for ease of handling while a black light was used in a dimly lit room to increase visibility of the fluorescent mark.

The jet injection marking method had 0% (CI: 0% - 31%) mortality and all fish in the experiment burrowed (100%, CI: 69% - 100%). However, mark retention was low (40%, CI: 12% - 74%) and two of the four marks considered visible were only detected when using a black light because only a small amount of the ink was retained. The diameter

of the jet injector was larger than the width of the caudal fin, making it difficult to successfully aim the injector and mark larvae while they were moving. Although mortality was not observed, the high-pressure of the injector caused tissue damage where the mark was administered on two fish. Tissue damage may have occurred because larval lampreys are soft bodied with only a small amount of surface area to properly administer the mark. Our study showed jet injection marking has limitations when used on unanesthetized larval lamprey; however, future studies may show this technique is more effective on larval lamprey that are anesthetized.

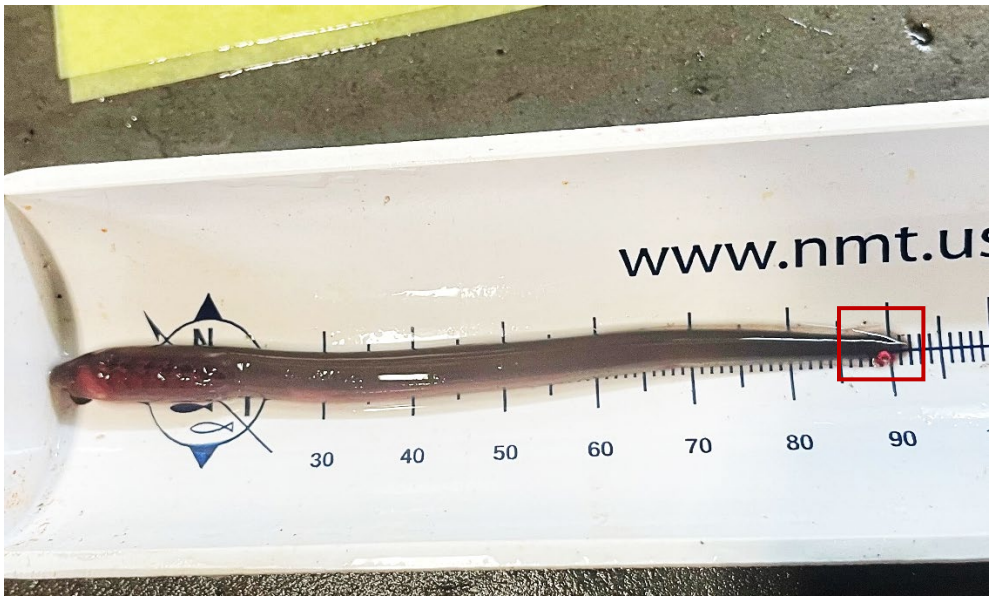


Figure 3. Larval lamprey on Day 2 of the jet injector experiment. The injector tool caused tissue damage (outlined in red) where administered on the caudal fin. Photo Credit: Kayla Kelley (USFWS).

None of three methods we examined proved to be effective methods for batch marking larval Pacific Lamprey. Bismarck Brown was highly visible (100%) but had high mortality rates (70% -100%) at both examined dilutions. Future studies with lower concentration dilutions may show different results and fewer mortalities. If proven to be a non-toxic method for dyeing fish, the high visibility of Bismarck Brown could be an effective batch marking technique. Although dyeing with fluorescein resulted in no mortalities, it did not leave a visible mark on larval Pacific Lamprey. Concentrations used were the same dilution as other fish dyeing studies. Lampreys have no calcified tissues and thus fluorescein may be ineffective as a dye regardless of concentration or duration. Jet injection, an effective marking method for other species, caused tissue damage to two of our study fish, and had low visibility (40%). Given the tissue damage, low visibility, and difficulty applying this marking method (e.g., softer tissues and less surface area), jet injection was not shown to be a useful marking method for unanesthetized larval lamprey.

Table 1. Mark retention, mortality, and tissue damage of marked larval Pacific Lamprey after one day of holding. Results are shown for each marking experiment.

Mark	Total Length	Total N	Burrowed	Visible Mark	Tissue Damage	Mortalities
Bismarck Brown (1:7,500)	< 90 mm	5	0	5	0	5
	≥ 90 mm	5	0	5	0	5
Bismarck Brown (1:15,000)	< 90 mm	5	2	5	0	3
	≥ 90 mm	5	1	5	0	4
Fluorescein	< 90 mm	5	5	0	0	0
	≥ 90 mm	5	5	0	0	0
Jet Injected Ink	< 90 mm	5	5	2	1	0
	≥ 90 mm	5	5	2	1	0

Figure 4. Experimental fish in buckets after dyeing placed into a holding container. Photo credit: William Simpson (USFWS).

References:

Beamish, R.J., and J.H. Youson. 1987. Life history of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the strait of Georgia. Canadian Journal of Fisheries and Aquatic Sciences 44:525–537.

Briand, C., D. Fatin, E. Feunteun, and G. Fontenelle. 2005. Estimating the stock of glass eels in an estuary by mark-recapture experiments using vital dyes. Bulletin Français de la Pêche et de la Pisciculture 378-379:23–46.

Clemens, B.J., R.J. Beamish, K.C. Coates, M.F. Docker, J.B. Dunham, A.E. Gray, J.E. Hess, J.C. Jolley, R.T. Lampman, B.J. McIlraith, M.L. Moser, M.G. Murauskas, D.L.G. Noakes, H.A. Schaller, C.B. Schreck, S.J. Starcevich, B. Streif, S.J. van de Wetering, J. Wade, L.A. Weitkamp, and L.A. Wyss. 2017. Conservation challenges and research needs for Pacific Lamprey in the Columbia River Basin. Fisheries 42:268–280.

Close, D.A., M.S. Fitzpatrick, and H.W. Li. 2002. The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19–25.

Deacon, J.E. 1961. A staining method for marking large numbers of small fish. The Progressive Fish-Culturist 23:41-42.

Dietrich, J.P., and R.A. Cunjak. 2006. Evaluation of the impacts of Carlin tags, fin clips, and Panjet tattoos on juvenile Atlantic Salmon. North American Journal of Fisheries Management 26:163–169.

Ewing, R.D., B.P. McPherson, and T.D. Satterthwaite. 1990. Effects of varied rearing temperatures and feeding regimes on retention of Bismarck Brown Y stain in alevins of Chinook Salmon. The Progressive Fish-Culturist 52:231–236.

Gaines, P.C., and C.D. Martin. 2004. Feasibility of dual-marking age-0 Chinook Salmon for mark–recapture studies. North American Journal of Fisheries Management 24:1456–1459.

Garner, B., C.L. Webb, S. Gale, and W. Fetzer. September 2019. An application of Bismarck brown Y dye as a short-term biological marker. In American Fisheries Society & The Wildlife Society 2019 Joint Annual Conference. AFS. Can be downloaded from (https://www.researchgate.net/publication/341056381_An_application_of_Bismarck_Brown_Y_dye_as_a_short-term_biological_marker).

Gilbert, E.I., S.L. Durst, T.A. Diver, H. Mello, N.G. Bertrand, and N.R. Franssen. 2020. Efficacy of fluorescent calcein mark retention and identification in endangered Colorado pikeminnow *Ptychocheilus lucius*. Fisheries Management and Ecology 27:258–269.

Hanson, K.C., and J.M. Barron. 2017. Evaluation of the effects of marking Pacific Lamprey ammocoetes with visual implant elastomer, coded wire tags, and passive integrated transponders. Transactions of the American Fisheries Society 146:626–633.

Hess, J.E., R.L. Paradis, M.L. Moser, L.A. Weitkamp, T.A. Delomas, and S.R. Narum. 2021. Robust recolonization of Pacific Lamprey following dam removals. Transactions of American Fisheries Society 150:56–74.

Hill, M.S., and C.J. Quesada. 2010. Calcein mark retention in chinook salmon and steelhead fry in artificial and natural rearing environments. North American Journal of Fisheries Management 30:1370–1375.

Meeuwig, M.H., A.L. Puls, and J.M. Bayer. 2007. Survival and tag retention of Pacific Lamprey larvae and macrophthalmia marked with coded wire tags. North American Journal of Fisheries Management 27:96–102.

Moffett, I., W. Crozier, and G. Kennedy. 1997. A comparison of five external marks for Atlantic salmon, *Salmo salar* L. Fisheries Management and Ecology 4:49–53.

Moser, M.L., A.D. Jackson, R.P. Mueller, A.N. Maine, and M. Davisson. 2017. Effects of passive integrated transponder (PIT) implantation on Pacific Lamprey ammocoetes. Animal Biotelemetry 5:1, DOI 10.1186/s40317-016-0118-3.

Noga, E.J., and P. Udomkusonsri. 2002. Fluorescein: a rapid, sensitive, nonlethal method for detecting skin ulceration in fish. Veterinary Pathology 39:726–731.

Pitsch, M., W. Hiemann, G. Burghardt, and M. Heynen. 2021. Colour marking of small fish with a marking stand for Dermojet. MethodsX 8:101510.

Silver, G.S, C.W. Luzier, and T.A. Whitesel. 2009. Detection and longevity of cured and uncured visible implant elastomer tags in larval Pacific Lampreys. North American Journal of Fisheries Management 29:1496–1501.

Stone, J., M. McGree, and T.A. Whitesel. 2006. Detection of uncured visible implant elastomer tags in larval Pacific lampreys. North American Journal of Fisheries Management 26:142–146.

Wang, C.J., and H.A. Schaller. 2015. Conserving Pacific Lamprey through collaborative efforts. Fisheries 40:72–79.

Ward, F.J., and L.A. Verhoeven. 1963. Two biological stains as markers for Sockeye Salmon fry. Transactions of the American Fisheries Society 92:379–383.

Wang, C.J., H.A. Schaller, K.C. Coates, M.C. Hayes, and R.K. Rose. 2020. Climate change vulnerability assessment for Pacific Lamprey in rivers of the Western United States. Journal of Freshwater Ecology 35:29–55.