Development of optimal methods for collection, transportation, holding, handling, and tagging of juvenile American shad

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Abstract American shad (Alosa sapidissima) are an anadromous fish species native to North America that have an extensive range, but their populations are declining. Acoustic telemetry can play a vital role in better understanding the behavior and survival of this sensitive species, but successfully handling and tagging juvenile American shad can be challenging. We conducted several experiments to determine the best methods for collecting, transporting, holding, and tagging juvenile shad. Minimizing out-of-water handling and the use of a saltwater treatment during collection increased 24 h survival from 78 to 99% after transport. Saltwater was also fundamental in keeping tagged shad alive overnight. Shad as small as 50 mm, were implanted with a dummy acoustic transmitter using a pectoral incision method with no suture. In a 60 d holding evaluation, the tagged fish survived at a rate comparable to their non-tagged counterparts (81.5% for tagged, 70% for untagged). Tagged and untagged shad also had similar survival when exposed to a tank of predators. The results are important for improving conservation efforts for small, sensitive species of fish, like American shad.

Keywords Acoustic transmitter · Alosa · Saltwater · Sensitive species · Transport

Introduction

American shad (*Alosa sapidissima)* are an anadromous fish species native to the East Coast of the United States. Shad populations have been declining, in part, because of reduced habitat availability, unsustainable adult mortality rates in some stocks, and increased number of hydropower dams that block upstream and downstream migrations (ASMFC 2009; Dadswell and Rulifson 1994; Limburg and Waldman 2009). Because of their cultural and economic value, efforts are being made to restore American shad habitat and populations all along their native range from Maine to Florida. There are more than 100 hydropower facilities in the United States located in the native ranges of American shad whose operating licenses will expire during the next 10 years (FERC 2022). Passage and survival studies where native shad populations occur will be an important consideration for relicensing these facilities. For example, the Connecticut River Shad Management Plan requires 95% passage within 24 h (h) and 95% passage survival for juvenile shad entering the forebay region of a dam (CRASC 2020). However, successful tagging

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techniques to assess the passage and survival of American shad are lacking.

A common challenge with tagging juvenile American shad is survival, as studies have shown high mortality rates. In one study, the authors reported 80% mortality of juvenile shad (mean length: 93 mm) tagged with radio-frequency transmitters within the first two days and 98% mortality within seven days post-tagging (Kleinschmidt et al. 2016). They also noted irregular swimming behavior for most tagged fish, with fish listing to one side (tag side down). Poor survival (28%) of smaller juvenile American shad (62–82 mm) implanted with acoustic transmitters was also observed in previous studies (Deters et al., unpublished data). For larger American shad (108–145 mm) tagged with external radio transmitters, Mathur et al. (2018) found no short-term impacts (< 24 h) for juvenile American shad, and the tags were deemed suitable for shad > 125 mm. A caveat to these short-term evaluations includes the uncertainty associated with obtaining adequate control groups and holding for > 48 h to evaluate delayed mortality and/or tag loss (CRASC 2020). The trend of poor post-tagging survival for American shad shows there is a need for improved tagging procedures to achieve accurate and unbiased survival estimates for this sensitive species.

In addition to tagging challenges, another known challenge of using American shad and other alosines for research purposes is their sensitivity to handling and transport (Bolland et al. 2019; Chittenden 1971; Shrimpton et al. 2001). Several studies have tried to determine the optimal methods for American shad transport with varying degrees of success. Raquel et al. (1989) found that handling and trucking mortalities were consistently higher for American shad than for the other five species in their study, including Chinook salmon (*Oncorhynchus tshawytscha)*, steelhead *(Oncohynchus mykiss)*, striped bass *(Morone saxatilis)*, threadfin shad *(Dorosoma petenense)*, and white catfish *(Ameiurus catus*). Sykes (1950) theorized that 100% mortality of juvenile shad during a 10-h long transport was due to several factors, including water agitation during travel, constant spray from a water pump, the use of freshwater, and increased water temperature. After addressing these factors, they achieved 100% survival in two subsequent transports. Shrimpton et al. (2001) found migrant juvenile American shad held in 32 parts per thousand (ppt) saltwater were less susceptible to stressors than migrants held in freshwater, which exhibited a heightened sensitivity to acute stress. To alleviate the stressors and loss associated with transport, many researchers have made efforts to collect, tag, and release fish at or near their study site.

Challenges in successfully handling, transporting, and tagging sensitive species such as American shad have resulted in knowledge gaps about their migration, behavior, and habitat use. The ability to track fish movement through hydropower structures would greatly advance our understanding of passage and survival rates for American shad. This would support more informed management decisions for existing hydroelectric facilities and better designs for new systems that minimize impacts to fish species and life stages not previously studied. To help improve the ability to track juvenile shad, Pacific Northwest National Laboratory (PNNL) developed a new acoustic micro-transmitter suitable for implantation in American shad and other fish species with similar compressiform body types. As new transmitter technology is developed, understanding the effects of the transmitter and the tagging process is imperative to minimize bias and accurately assess passage and survival. Potential transmitter effects include impacts to fish growth, survival, and behavior. The added burden from the tag weight can impact swimming ability, buoyancy, and the ability to avoid predation. The generally accepted rule is that the tag should weigh less than 2% of the fish's weight in air (Winter 1996), but some studies have shown a tag burden up to 7% of the fish's weight did not cause adverse effects in long-term survival of juvenile salmonids (Brown et al. 2010; Chittenden et al. 2009). However, species-specific variations can occur and sensitive species such as American shad may have a lower tag burden tolerance.

The goal of this research was to provide best practice guidelines that minimize the negative effects associated with handling and tagging juvenile American shad. The objectives of this laboratory study were: 1) to identify the best methods and conditions for transporting, holding, rearing, and handling American shad, 2) to evaluate five micro-transmitter prototypes and associated tagging techniques, and 3) to establish a tagging protocol that maximizes survival and tag retention while minimizing any potential tag effects related to predator avoidance. The development of handling and tagging guidelines will allow meaningful and unbiased field tests to address the knowledge gaps regarding American shad survival and behavior, which will also help inform future re-licensing efforts for hydropower dams located within the shad's native range.

Methods

The following evaluations took place over two years of juvenile American shad fall migration. These procedures were reviewed and approved under protocols 2020-02 and 2021-08 by PNNL's Institutional Animal Care and Use Committee, which complies with the U.S. National Research Council's Guide for the Care and Use of Laboratory Animals. PNNL is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Transport evaluation

Two separate transport events were conducted in the fall of 2020 using modifications of techniques employed in other juvenile shad research (Castro-Santos and Haro 2015; Chittenden 1971; Sykes 1950). The first transport event occurred on September 8, 2020, when approximately 2,150 juvenile American shad were collected from the McNary Dam juvenile bypass facility and transported to the Aquatic Research Laboratory (ARL) at PNNL in Richland, Washington (~50 min transport duration). The second transport event occurred on September 24, 2020, when approximately 1,000 juvenile shad were collected from McNary Dam juvenile bypass facility and transported to the ARL.

During the first transport event, shad were lightly sedated (stage 2–3; Summerfelt and Smith 1990) with a 60 parts per million (ppm) dose of tricaine methanesulfonate (MS-222) in the McNary Dam sampling trough. They were netted from the water and placed in another net that was partially submerged in the trough. After several minutes in the net, the fish were transferred to a bucket (19 L) on a tared scale to get an estimate of the total weight of the fish collected. Then the fish were transferred in-water from the bucket into the insulated fiberglass circular tanks (1.1 m diameter, 0.7 m deep) supplied with Columbia River water (20 °C). The water was treated with commercial sea salt (Instant Ocean, Blacksburg, Virginia) at a concentration of 7.5 ppt (measured using a portable meter; Extech EC-400, Boston, MA).

Two trucks, each containing one circular tank, were used to compare transportation methods. One tank was supplied with an internal water pump with two outlets to create a circular flow (pump method). The other tank used an air-lift system with one outlet and an external air pump and air stone to create the circular flow (air-lift method; Fig. 1). Both tanks also were supplied with compressed oxygen via an air stone located at the base of the tanks. The two tanks were outfitted with a circular piece of 3.8 cm-thick foam, which was placed on the water surface under the tank lid to reduce water agitation during transport. Water temperatures ranged from 19 to 20 °C and dissolved oxygen was 92–123%.

Upon arrival at the ARL, fish were guided into buckets with a net and placed into four circular holding tanks (1.2 m diameter, 0.6 m deep) using a water-to-water transfer method. Ambient water from the Columbia River was used for all fish holding and testing in this study. Unless stated otherwise, flow-through systems were set up to provide approximately 3–4 tank exchanges per hour. Fish from both transport treatments were divided into two holding tanks per treatment. All tanks were adjacent to each other, and fish were exposed to a similar, natural photoperiod. An attempt was made to enumerate the fish transferred into each tank, but the main goal was to make water-to-water transfers while keeping the stress level as low as possible. Final estimated numbers of fish transferred into each tank were: 630 and 488 in the water pump treatment tanks, and 592 and 448 in the air-lift treatment tanks. Tanks were checked daily, and all mortalities were recorded. Fish were fed a maintenance ration of BioVita MASH (BioOregon, Longview, Washington) at approximately 1–2% body weight per day, beginning the day after transport. Fish survival in each holding tank was evaluated from truck departure at McNary Dam through 7 days (d) holding at ARL.

During the second transport event, juvenile shad were collected from McNary Dam (19 °C water temperature) and transported to PNNL (18 °C). In an effort to further improve transport survival, two modifications were made to the original (i.e., first transport)

Fig. 1 Photographs of the **a** collection trough at McNary dam with lightly anesthetized American shad and a bucket of saltwater, **b** the transport tank in the bed of a pickup truck, and inside of the transport tank showing the water pump setup (right, inset), and **c** the air-lift setup used at the aquatic research laboratory. In the holding evaluation, compressed air was used to aerate and create current velocity in the static water tanks

collection methods at McNary Dam to further reduce stress on the fish: a softer dip-netting material (0.44 mm nylon mesh) compared to typical aquarium nets was used to collect fish from the trough, and the length of time fish were held in the net was greatly reduced (from > 1 min to ≤ 1 s). The previous truck transport methods (pump and air-lift) were repeated. At the ARL, fish were held in two separate test conditions (4 tanks; each 1.1 m diameter). Two tanks contained water with a salinity of 5–8 ppt and two tanks contained unsalted freshwater. Fish from each transport method were split between the saltwater and freshwater tanks. For this manuscript, the term saltwater will be used to signify water treated with salt to a concentration of ~ 7.5 ppt; this term easily differentiates from freshwater.

Holding evaluation

Four treatments were tested for the holding evaluation: air-lift transport with saltwater (salt-air) or freshwater holding (fresh-air) and pump transport with saltwater (salt-pump) or freshwater holding (fresh-pump). Study fish were held in four tanks (1.1 m diameter) by treatment group with salinity ranging from 5.7 to 7.7 ppt for saltwater tanks and < 1 ppt for freshwater. An air-lift system (similar to that described in the transport evaluation) was used in all tanks at the ARL to aerate the static water (98.1–108% dissolved oxygen; 16.8–19.3 °C) and to create circular current velocity within the tanks (Fig. 1). The tanks were partially covered with netting and black fabric shades to provide cover and minimize stress from other activities in the lab during the holding evaluation period. Fish were exposed to a simulated natural photoperiod and tanks were checked daily for mortalities. Waste was siphoned from tanks once or twice daily as needed. Fifty percent water changes occurred approximately every other day or as needed when the concentration of ammonia approached 2 ppm (range 0–2 ppm during study). Fish were fed a maintenance ration every other day throughout the study (BioVita MASH; ~ 1–2% biomass per feeding) and they were monitored for 14 days. Beginning on day 7, tail-rot was observed on mortalities that were removed from the fresh-air tank and on day 13; fungus was observed on several mortalities taken from this tank.

Handling evaluation

The handling evaluation was performed to identify an optimal handling method to minimize stress to juvenile American shad and maximize survival. The handling evaluation was conducted under the assumption that if American shad die during or after handling without undergoing tag implantation, they would also perish after tag implantation. Conversely, if the fish survive during or after handling without undergoing tag implantation, they would have a higher likelihood of surviving after tag implantation. Thus, the handling method with the highest survival is likely to result in higher survivorship when fish are implanted with transmitters.

We were unable to find an accepted standard handling practice for juvenile American shad in the grey or peer-reviewed literature. Fine details of handling methods such as techniques used to move fish from a holding tank to an anesthetic bath, are likely omitted from a publication. For this evaluation, we compared survival of shad that were subjected to an out-of-water handling treatment (Out) to shad that were subjected to in-water handling treatments (In) and to those subjected to a combination handling treatment of both in-and-out-of-water (In/Out; Table 1).

The two saltwater tanks used in the holding evaluation from fish transported on September 24, 2020, were used as the source tanks for the handling evaluations (Fig. 2), ensuring that all test fish experienced the same pre-test rearing conditions. Fifteen fish from each source tank were used for each treatment. On October 13, 2020, four groups were evaluated using three dosing levels of anesthesia and a control

Table 1 Stepwise procedure and sample size by treatment group (MS-222 light dose, heavy dose, no dose, and controls) and treatment (in water, in-and-out of water, and out of water). The stepwise handling procedures are described more fully within the text

Group	MS-222 light dose			MS-222 heavy dose			No dose		Control	
Treatment	In	In/out	Out	In	In/out	Out	In	Out	In	Out
Step 1—transfer	In	In	Out	In	In	Out	In	Out	In	Out
Step 2—anesthesia	In	In	Out	In	In	Out	In	Out	None	None
Step 3—measure	In	Out	Out	In	Out	Out	None	None	None	None
Step 4—transfer	In	None	None	In	None	None	None	None	None	None
Step 5—transfer	In	Out	Out	In	Out	Out	In	Out	In	In
Sample Size	30	30	30	30	30	30	30	30	30	30

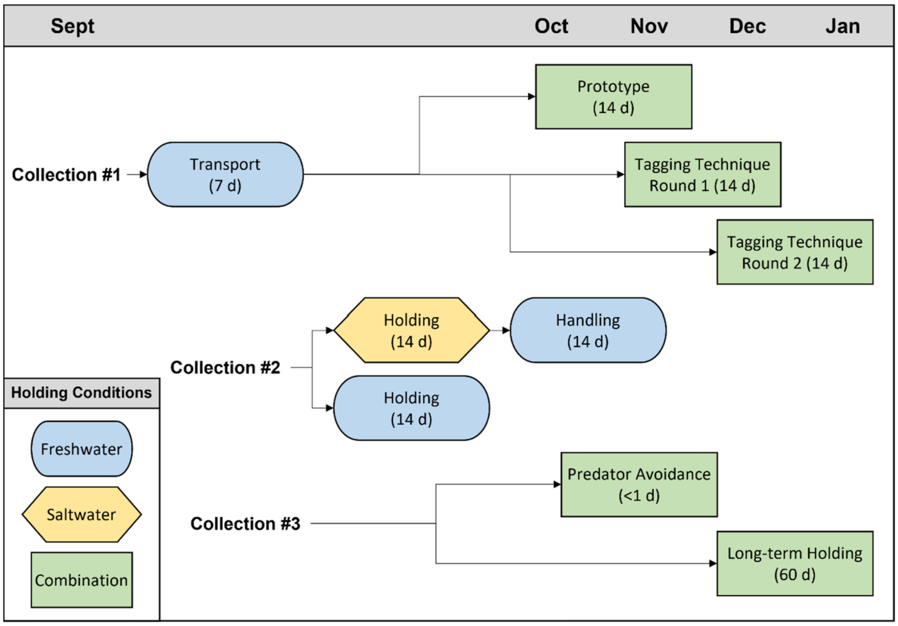


Fig. 2 Flowchart, timeline, and duration of all evaluations completed for this study, including shad held in freshwater and saltwater, and shad held first in saltwater and then in freshwater during the evaluation period

group. The three different MS-222 doses were: light (60 ppm to stage 2 anesthesia: deep sedation), heavy (120 ppm to stage 4 anesthesia: total loss of equilibrium), and no dose (Summerfelt and Smith 1990). An anesthesia-free dose (no dose) was a viable treatment option because no anesthetic is used in some adult shad telemetry studies (Breine et al. 2017; Eakin et al. 2017; Moser et al. 2000; Smith et al. 2009). One of three handling options (completely in water [In], completely out of water [Out], or not handled [None]) were used for each of the five steps of the handling process (Table 1):

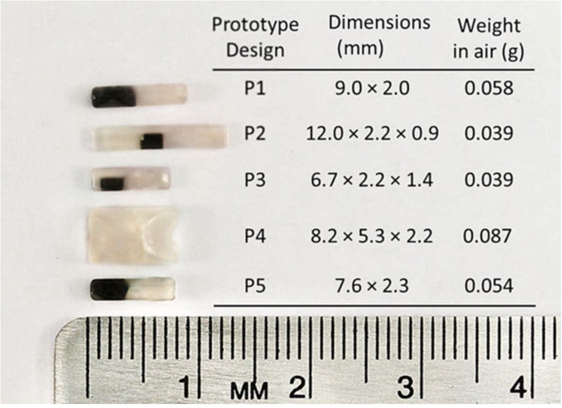
- *Step 1* Transfer fish from pre-treatment holding tank to treatment bucket (In or Out)
- *Step 2* Administer anesthesia (In, Out, or None)
- *Step 3* Measure length (In, Out, or None)
- *Step 4* Transfer fish from measuring board to cup of anesthesia (In or None)
- *Step 5* Transfer fish to post-treatment holding tank (In or Out)

The five steps were performed in duplicate with a bucket of 15 fish treated as a batch. Two batches, one from each source tank, for each treatment resulted in a total of $n = 30$ fish per handling treatment (Table 1). Overall, there were 10 treatments for the handling evaluations. Each group of treatment fish were in a different post-handling tank (189 L semi-square shape) and held for two weeks in freshwater. Fish were observed for 14 days and checked at least daily for dead or moribund fish.

The results of the handling evaluation identified the MS-222 heavy dose In/Out as the method with the highest survival. As such, a simplified variation of the MS-222 heavy dose In/Out method was used for the remainder of the evaluations and tagging process. The simplified variation steps consisted of guiding approximately 30 shad with a net from the holding tank into a bucket of water, leaving approximately 3 L of water in the bucket for a final fish density of approximately 12–40 g/L. Then, sea salt was dissolved in another bucket with approximately 3 L of water; the saltwater was added to the bucket of fish to obtain a saline concentration of approximately 7.5 ppt. Next, a 120-ppm dose of anesthetic saltwater (7.5 mL of 80 g/L MS-222, 7.5 mL of 80 g/L sodium bicarbonate, and sea salt) was prepared in another bucket. Using a 0.4 mm nylon mesh dip-net, one fish was netted out of water from the source bucket of fish and placed into the anesthetic bucket. Once the fish reached stage four of anesthesia (Summerfelt and Smith 1990), it was removed from the bucket with a nitrile-gloved hand, placed on a wet measuring board, and then placed in a small water-filled container to record fork length and weight. To implant or attach the tag, the fish was placed in a small container of saltwater and tagged using the procedures described below.

Prototype evaluation

The prototype acoustic transmitter designs were conceptualized before the technological development of the transmitters could be completed. The designs consisted of two options for the battery geometry (flat or cylindrical) and two options for the transducer geometry (flat or cylindrical) and several form factors. Dummy tags of the same dimension and weight as prototype transmitter designs, but without functional parts, were used for this study (Fig. 3). Prior to evaluating the prototype tags, tagging practice was conducted in early October 2020 at the ARL to refine the implantation techniques and attempt to keep shad alive for several days post-tagging. Initially, when



Prototype Design	Dimensions (mm)	Weight in air (g)
P1	9.0 × 2.0	0.058
P2	12.0 × 2.2 × 0.9	0.039
P3	6.7 × 2.2 × 1.4	0.039
P4	8.2 × 5.3 × 2.2	0.087
P5	7.6 × 2.3	0.054

Fig. 3 Acoustic micro-transmitter design options tested as part of the prototype (one design is not shown), tagging techniques (the first iteration of the P5 design is not shown), predator avoidance, and long-term holding evaluations. From top to bottom, transmitter designs P1, P2, P3, P4, and P5 are shown with a ruler in millimeters for scale. Prototype P4 is the only design for external attachment and was designed to be neutrally-buoyant; thus, the tag weight should be negligible to the fish in water

using freshwater, an oily and discolored residue was observed in the pre-tagging and anesthesia buckets; no fish survived tagging overnight. After switching to 7.5 ppt saltwater during the pre-tagging, anesthesia, and recovery periods, the tagged shad survived for up to 10 days.

Tagging for the prototype evaluations was completed on October 16, 2020, using shad transported on September 8. Five prototype design options were paired with four tagging locations to test eight unique transmitter-location treatments with 9–20 fish per treatment. Tagged groups were compared to an untagged control group. Fish were held in two 189 L semi-square tanks for 14 days. Post-tagging treatment, holding, and monitoring for the prototype evaluation followed the same methods described below. Results from this initial round of prototype testing were used to determine the best transmitter designs to pair with a single tagging location and method for the tagging techniques evaluation. Results were determined by surgeon experience and expertise, as well as biological observations, as opposed to statistical analyses.

Tagging techniques evaluation

Using the same established techniques from the prototype evaluation, a more robust tagging study was initiated on November 19, 2020, using the shad transported on September 8. Four of the transmitter design options from the prototype evaluation were tested against a fin-clipped control group with 40 fish per group (Table 2; Fig. 3). A new transmitter design, P5, was developed in late December. Testing was planned for early January 2021 but was delayed until mid-January due to a fungal infection that was remedied using a formalin treatment prior to testing. Once healthy, the P5 design was evaluated in these fish using the pectoral incision implantation method against a non-tagged control group on January 14, 2021 (Table 2). Dorsal fin clips were not used to identify the controls because tagged fish could be identified by the incision if a tag was expelled. As a result, the tagging techniques evaluation had six treatments that included five tag prototypes and a group of control (untagged) fish. Fish were held for 14 d to evaluate survival and tag retention. Four prototypes (P1–P4) and one group of control fish were evaluated in November 2020 and the fifth prototype (P5) was evaluated with a separate group of control fish in January 2021.

For all internal implants and external (dorsal) attachments, fish were placed in a groove on a soft piece of foam pad during the tagging process (Fig. 4). A gravity-fed tank delivered fresh river water over the fish's gills throughout the procedures. Pectoral and pelvic incisions were made with a #11 surgical blade (Oasis, Mettawa, Illinois). The pectoral incision was made on the fish's right side near the distal end of the pectoral fin in a vertical orientation to cut between myomeres. The tag was inserted posteriorly, and the blunt, back end of the blade was carefully used to fully insert the tag into the fish as needed. The pelvic incision was made roughly vertically between myomeres on the fish's left side several millimeters posterior to the distal end of the pelvic fins (Hollis 1948). The tag was inserted anteriorly, and the blade was used to fully insert the tag as needed. For the dorsal attachment method, the fish was placed ventral side down on the foam pad. Then, a 5–0 Monocryl suture with RB-1 needle (Ethicon, Somerville, New Jersey) was used to attach the tag just anterior of the dorsal fin using a $1\times1\times1\times1$ square knot (Deters et al. 2012). For the gastric tagging method, fish were held underwater in a bath of freshwater (Fig. 4). All but the snout remained underwater while lab tools were used to insert the tag into the mouth, esophagus, and finally the stomach (Castro-Santos and Vono 2013; Smith et al. 2009). Needle holders and forceps were disinfected with a hot bead sterilizer after each use, while the rest of the surgical tools were disinfected with a UV light sterilizer (Millipore, Billerica, Massachusetts; Walker et al. 2013).

Shad were held in one of two circular tanks (1.1 m diameter), for a total of 100 fish per tank in November 2020 with equal numbers of each treatment in both tanks. In January 2021, 80 fish were held in one circular tank (1.1 m diameter). All fish were held in a static tank of aerated saltwater for the first two days post-tagging to maximize survival. On the third day, the tanks were switched to flow-through ambient freshwater for the remainder of the 14-d evaluation in November 2020 (9.1–16.1 °C) and January 2021 (10.7–14.7 °C). Fish length ranged from 50 to 80 mm (November 2020) and 51–75 mm (January 2021; Table 2). Tanks were monitored daily for mortalities and expelled tags.

Table 2 Tagging and survival information for American shad implanted with the different prototype transmitters (tag type) for tagging evaluations in year one and year two of the study. The evaluations are listed by tag date: tagging techniques round 1 (11/20/2020), tagging techniques round 2 (1/14/2021), predator avoidance (Nov/Dec 2021), and long-term holding tank B (1/14/2022). Tag location for controls is Not Applicable (NA) and they are listed as having a fin clip (FC) or having no fin clip (NC). Note, tag type P5 in the 14-day evaluation in January 2021 was a different weight than P5 used from November 2021 through March 2022 and P4 was a neutrally buoyant design

Tag date	Tag type	Tag location	*N*	Fork length (mm)		Tag weight (g)	Tag burden (%)	Eval. period (d)	Survival (%)	Time to tag/clip (s)
				Range	Average ± SD					
11/20/2020	P1	Gastric	40	50–76	60 ± 6.0	0.058	1.5—5.2	14	45	12
	P2	Pectoral	40	50–78	60 ± 7.3	0.039	1.0—3.2	14	80	23
	P3	Pelvic	40	50–70	58 ± 5.3	0.039	1.0—4.1	14	55	26
	P4	Dorsal	40	50–80	61 ± 6.8	0.088	0	14	60	57
	Control	NA (C)	40	50–80	59 ± 5.7	NA	0	14	92.5	14
1/14/2021	P5	Pectoral	40	51–73	63 ± 5.1	0.044	1.5—5.6	14	75	26
	Control	NA (NC)	40	53–75	63 ± 4.6	NA	0	14	87.5	31
Nov/Dec 2021	P5	Pectoral	86	55–100	80 ± 8.2	0.058	0.6—4.1	< 1	23	-
	Control	NA (NC)	85	56–97	79 ± 7.3	NA	0	< 1	27	-
1/14/2022	P5	Pectoral	27	69–105	90 ± 7.1	0.058	0.5—1.5	60	81.5	-
	Control	NA (NC)	10	85–95	91 ± 3.1	NA	0	60	70	-

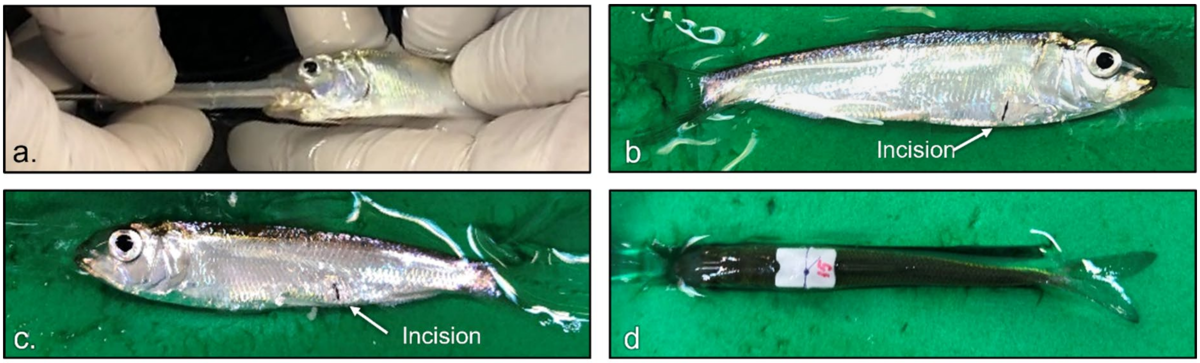


Fig. 4 The four tagging technique methods used in the evaluation were: **a** gastric implantation, **b** pectoral incision, **c** pelvic incision, **d** and dorsal attachment with suture

Additional evaluations with shad transmitter design P5

The second year of the laboratory study began in the fall of 2021, when approximately 1150 juvenile American shad were collected from McNary Dam between September 20 and 24, 2021, at a water temperature of 19 °C, and transported using the pump method described in the transport evaluation. Due to efforts to reduce MS-222 disposal at the McNary Dam collection facility, fish were not anesthetized during the sorting process. These fish were also larger on average than fish collected in 2020 (55–100 mm FL; average 80 mm) and the 1 d transportation survival ranged from 86.2 to 89.9%. Fish in the source tanks (1.2 m diameter, 0.6 m deep) were held in freshwater until they were used for the long-term and predator avoidance evaluations.

Prior to the initiation of year two evaluations, preliminary post-tagging holding and predation tests were conducted to confirm that the post-tagging saltwater holding period could be reduced from 2 to 1 d without affecting shad survival. This holding time is more representative of field studies conducted with migrating juvenile fish (e.g., Harnish et al. 2020). All subsequent testing in year two used a 1-d saltwater holding period following implantation.

Long-term holding evaluation

The long-term holding evaluation had two treatments: one tag prototype and one group of control fish. Shad were tagged with prototype P5 using the pectoral tagging procedure described in the tagging techniques evaluation. The dimensions and weight of P5 changed slightly from January 2020 to November 2021 (Table 2). The long-term evaluation was terminated after a turbid water event resulted in high mortality of tagged and control fish. Because the source tanks of shad were seemingly unaffected by the event, the evaluation was completed with the remaining fish. There were insufficient numbers of shad remaining to conduct the study as designed ($n = 100$ per group), so sample sizes were reduced to complete the long-term holding evaluation. On January 14, 2022, 54 tagged and 19 control fish were placed into two replicate circular tanks (1.1 m diameter) with approximately equal fish density and number of fish per treatment (Table 2). The covered, static tanks (approximately 8 ppt) were aerated using the air-lift system described in the holding evaluation. After 24 h, the tanks were switched to flow-through freshwater. Fish were held at the ARL for 60 d to evaluate longer term survival (i.e., survival beyond the expected tag life of P5, which is expected to be ~ 30 d with a 5-s pulse rate interval), growth, and tag retention.

Tanks were checked twice daily for mortalities and expelled tags. If a fish appeared moribund (i.e., loss of equilibrium, erratic swimming behavior, etc.), it was removed from the tank, euthanized, and recorded as a mortality. Fork length was recorded and tags were removed from mortalities to identify treatment, individual, and to evaluate tag retention. Water temperature ranged from 14.9 to 17.0 °C throughout the study. Fish were fed on a maintenance ration of 1–2% body weight every other day throughout the study (BioVita MASH). After 60 d, all remaining fish were euthanized and their lengths, weights, and tag presence were recorded. Survival of tagged fish was defined by survival and tag retention throughout the 60-day period.

Predator avoidance evaluation

Largemouth bass (*Micropterus salmoides*) were chosen as the predator to use in the evaluation of predator avoidance because they are readily available in the Pacific Northwest and are known to utilize juvenile clupeids in their diet (Weinersmith et al. 2019). A total of 25 bass (approximately 220 mm on average) were acquired from Santiam Valley Ranch Aquaculture in Oregon and transported to PNNL on September 28, 2021. Largemouth bass were acclimated to ambient water conditions at PNNL for one week. After that, water temperatures were adjusted up to 2 °C per day and then held at the same temperature as the American shad. Largemouth bass were trained as predators by feeding one to two American shad per bass approximately every 2–3 days prior to the study. No artificial refuge or shelter was provided for the shad.

One day prior to testing, shad were anesthetized, measured, and tagged with the P5 prototype tag using the pectoral incision location. Control fish were anesthetized and measured. Five controls and five tagged fish were placed in each bucket, with 2 buckets per predator avoidance trial, and allowed to recover in saltwater for 1 d (Fig. 5). On the morning of the trial, buckets were checked for dead or moribund fish and if found, those fish were removed from testing. Tagged fish ranged in size from 55 to 100 mm and controls were 56–97 mm (Table 2). A total of 171 shad were tested.

Nine predator avoidance trials were conducted from November 4 through December 22, 2021, at a water temperature of 16 °C (±2 °C) in all fish holding tanks, including the shad pre-tag freshwater tank, shad post-tag saltwater tank, and freshwater test tanks. Two trials could be run in parallel because two circular test tanks were used (1.8 m diameter, 53 cm water depth) with 10 bass in each tank. Bass were used for multiple trials; however, a period of at least 3 d between trials was required to ensure predators were not overfed or uninterested in feeding during a trial. To start the trial, two buckets of shad (approximately 10 tagged and 10 untagged control

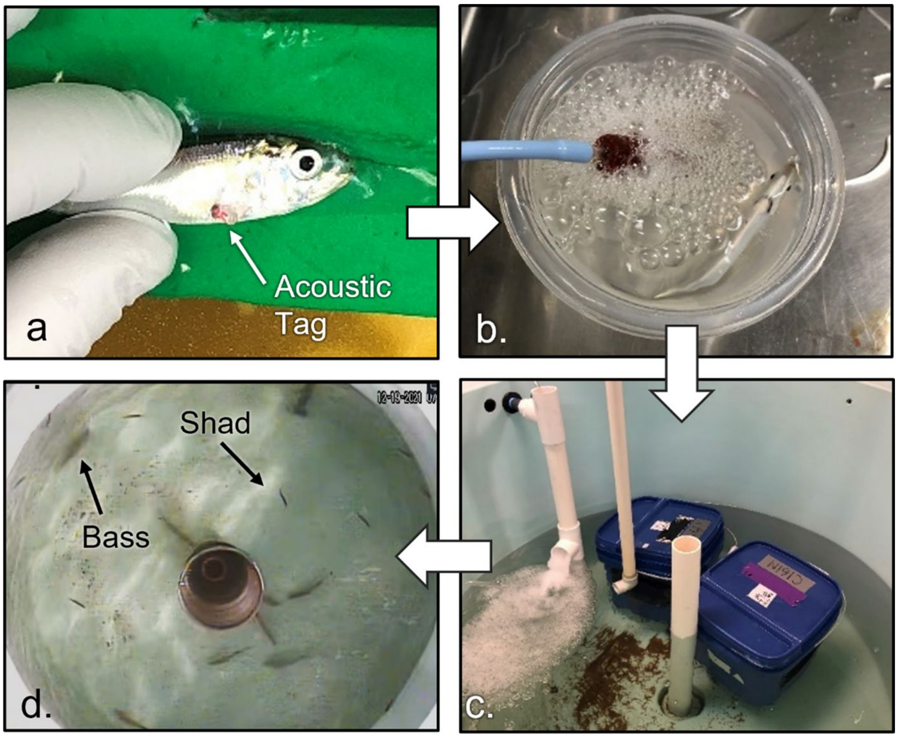


Fig. 5 Steps of the predator avoidance evaluation, including **a** tag implantation, **b** recovery from anesthesia, **c** overnight holding in saltwater, and **d** largemouth bass predator tank

fish) were simultaneously added to the bass tank using a water-to-water method. At the end of the trial, the remaining shad were removed from the tank and identified for evaluation. Trial duration ranged from 3 min to 2.1 h (median: 7.6 min).

To observe testing without disturbing fish behavior, a video camera (CCTV Sony CSP-750IR24, Cherry Hill, New Jersey) was affixed to a modular frame for overhead monitoring and recordings were made to a digital video recorder (CCTV 960H Cherry Hill, New Jersey). Observations from the live video feed were made at 15 min intervals and observations at the tank were made every hour until the end of the trial. Trials were terminated when approximately 50% of the prey were consumed (Anglea et al. 2004; Janak et al. 2012; Thompson et al. 2014). Due to the sensitivity of juvenile shad, any fish that was seriously injured (e.g., open wound, loss of equilibrium, continuous erratic swimming, and or lying on the bottom of the tank) was classified as wounded. Only fish that were not wounded, not killed, and not eaten were categorized as surviving the predation event.

Statistical analyses

The objective was the same for each evaluation in this study: to identify differences between treatments. In evaluations that used multiple test tanks for the same treatments, two-sided Fisher's exact tests were used to evaluate tank effects. If no significant difference was detected, fish from both tanks were combined for further analyses. Similarly, to compare between and among treatments, a two-sided Fisher's exact test was used to test whether one treatment performed worse than another treatment. If a significant difference was detected ($P \leq 0.050$), pairwise comparisons were performed using Fisher's exact tests to identify which treatments were significantly different from each other. For all pairwise comparisons, the P-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method at a 5% cut-off.

Treatment groups were evaluated by the proportion of fish that survived each treatment. The transportation, holding, and handling evaluations did not use tagged fish because survival was used to compare the methods; it was not a comparison of tagged to untagged fish. Study durations varied by evaluation. The transportation evaluation compared survival after 7 days, the holding evaluation at 7 and 14 days, handling evaluation at 14 days, tagging techniques at 14 days, long-term holding evaluation at 60 days, and predator avoidance at the trial end (<1 day). The holding evaluation was assessed at two intervals to compare survival among treatments for different holding durations because of a disease outbreak in one of the test tanks. For the handling evaluation, a comparison of 14-d survival for the Out, In/Out, and In handling treatments was performed first to understand if handling treatments differed before comparing the dosage levels (light, heavy, no dosage).

The purpose of the tagging techniques study was to evaluate fish survival among different tag implantation or attachment locations. Treatment fish in November were tagged in the gastric, pectoral, pelvic, or dorsal locations with prototypes P1, P2, P3, and P4, respectively. In January, treatment fish were also tagged in the pectoral location but with a slightly modified prototype of P2 (i.e., rounded edges of the tag to minimize potential internal irritation to the fish), called P5. Although both were implanted in the pectoral location, prototypes P2 and P5 were compared separately to determine if the design modification affected survival. Treatment fish from both tagging dates ($n = 40$ per prototype) were compared to each other and to the groups of control fish ($n = 80$), which were combined across tagging dates.

The long-term holding evaluation was conducted to assess 60-d survival and tag retention using a larger sample size of tagged fish and the best location identified from the tagging techniques evaluation. Survival in the tagging techniques and long-term holding evaluations used tagged fish to compare survival to untagged control fish. Survival for tagged fish in these evaluations was further defined as a fish that both survived and retained its transmitter. In a telemetry field study, a fish that expels its tag cannot be differentiated from a fish that dies, because in both instances, the acoustic signal from the transmitter stops moving. Thus, in the laboratory study, an expelled tag was recorded as a mortality. Finally, the purpose of the predator avoidance study was to evaluate the effect of the best identified tagging location from the tagging techniques evaluation on the performance of tagged fish exposed to predators, compared to their untagged counterparts.

For the tagging techniques evaluation, a mixed effects Cox proportional hazards model for censored survival data was fit by maximum likelihood using ‘survival’ and ‘coxme’ packages in R (Therneau 2000, 2022, 2023; R Core Team 2022) to evaluate the effect of tag type and location on 14- and 60-d survival. Treatment group (tag type/location, control) was a fixed effect in the model and tank was considered a random effect. Separate Cox proportional hazards models were fit to the data from the three different holding experiments. The first experiment included untagged controls, P1/gastric (tag type/location), P2/pectoral, P3/pelvic, and P4/dorsal tagged groups held for 14 days. The second experiment included untagged controls and fish implanted with the P5 tag in the pectoral region held for 14 days. The third experiment consisted of 60-d holding of untagged controls and fish tagged in the pectoral region with the P5 tag. Fish that survived to the end of the holding period were structured in the model as censored observations and fish that died or expelled their tag were included as complete observations. In addition, cumulative survival curves were compared between groups for the duration of each experiment.

Fish in the tagging techniques evaluation were smaller than fish in the long-term holding evaluation. Therefore, an analysis was conducted to determine whether fish size (i.e., FL) affected survival of implanted shad. Knowing whether or not size affected survival was important to ensure the difference in size didn’t bias the results of the tagging techniques evaluation. A generalized linear model (GLM) was fit to the 14-day post-tagging survival results from both the tagging techniques evaluation (fish implanted in the pectoral location using prototypes P2 and P5) and the long-term holding evaluation (pectoral location, prototype P5, and tank B only). The GLM incorporated a logit link function and Bernoulli error structure with terms for the intercept and fish size (Eq. 1):

$$(S) = \frac{1}{1 + \exp\left(-\left(\beta_0 + \beta_1 X\right)\right)} \tag{1}$$

where the estimated probability of survival and tag retention $P(S)$ is represented as a logistic function with intercept β_0 and regression coefficient β_1 for fish size (FL) fit to the binomial survival data, where fish that survived and retained their tag were assigned a 1 and fish that did not were assigned a 0. Applying this approach was determined to be appropriate by evaluating Pearson goodness-of-fit (GOF) test results for the model ($\chi^2 = 110.830$, $P = 0.458$).

Predator avoidance was also evaluated using GLMs with a logit link function (Pearson GOF: $\chi^2 \leq 170.785$, $P \geq 0.404$). The effect of fish size and treatment (tagged or untagged) on predator avoidance was evaluated by first fitting a full model that included terms for FL and treatment (T, tagged or untagged) (Eq. 2):

$$P(S) = \frac{1}{1 + \exp\left(-\left(\beta_0 + \beta_1 FL + \beta_2 T + \beta_3 FL \bullet T\right)\right)} \tag{2}$$

where the estimated survival probability $P(S)$ is represented as a logistic function with overall intercept β_0 and slope β_1, β_2,, which estimates the difference in intercepts between tagged and untagged fish, and an interaction term, $\beta_3 FL \bullet T$, which allows the slope to differ between tagged and untagged fish. This full model was fit to the binomial survival data, where fish that survived and were not wounded (hereafter referred to as survival) were assigned a 1 or otherwise a 0. The full model was then compared to all possible reduced models using Akaike’s information criterion (AIC) and likelihood ratio test (LRTs). The best-fitting model was identified as the model with the lowest AIC that significantly improved the fit over nested models containing fewer parameters as indicated by LRTs. In addition, the predictive potential of each model was evaluated using the area under the curve (AUC) metric (Hosmer and Lemeshow 2000). All analyses for this study were performed using R (R Core Team 2022).

Results

Transportation evaluation

Survival on day seven differed between tanks that held fish for the two pump treatments (Fisher’s test; $P = 0.048$); therefore, tanks remained separate for subsequent analyses. No difference in survival was observed between tanks for the air-lift treatment ($P = 0.392$), allowing tanks to be combined. A comparison of survival between pump and air-lift treatments indicated they were similar (Fisher’s test, $P = 0.116$; Table 3).

Table 3 Proportional analyses of survival for juvenile American shad by evaluation

Transportation– 14-day evaluation

Treatment	Dead	Alive	Total	Survival proportions	Fisher's test (P-value)
Pump (Tank A)	184	446	630	0.708	0.116
Pump (Tank B)	116	372	488	0.762	
Air-Lift (Tanks Combined)	271	769	1040	0.739	

Holding–7-day evaluation

Treatment	Dead	Alive	Total	Survival proportions	Fisher's test (P-value)
Salt-Air^{a}	1	252	253	0.996	***0.003***
Salt-Pump^{a}	1	253	254	0.996	
Fresh-Air^{b}	9	228	237	0.962	
Fresh-Pump^{a}	2	249	251	0.992	

Holding–14-day evaluation

Treatment	Dead	Alive	Total	Survival proportions	Fisher's test (P-value)
Salt-Air^{a}	4	249	253	0.984	***<0.001***
Salt-Pump^{a}	1	253	254	0.996	
Fresh-Air^{b}	48	189	237	0.797	
Fresh-Pump^{a}	2	249	251	0.992	

Handling–14-day evaluation–out, in/out, and in anesthesia comparison

Treatment	Dead	Alive	Total	Survival proportions	Fisher's test (P-value)
Out Anesthesia^{a}	39	80	119	0.672	***<0.001***
In/Out Anesthesia^{b}	7	53	60	0.883	
In Anesthesia^{a}	18	101	119	0.849	

Handling–14-day evaluation–in/out and in anesthesia comparison

Treatment	Dead	Alive	Total	Survival proportions	Fisher's test (P-value)
Light In/Out	4	26	30	0.867	0.964
Heavy In/Out	3	27	30	0.900	
None In	4	27	31	0.871	
Light In	5	25	30	0.833	
Heavy In	4	26	30	0.867	
Control In	5	23	28	0.821	

A P-value is significant at $\alpha = 0.05$ and is denoted by bolded and italicized text.

Significant pairwise comparisons are indicated with different superscript letters per evaluation.

Holding evaluation

On day seven, a significant difference in survival was observed among treatments (Fisher's test, $P = 0.003$; Table 3). The fresh-air treatment survival was 96.2% ($n = 9$ mortalities), which was significantly lower than the salt-air and salt-pump holding treatments, which had $\geq$ 99.6% survival ($n = 1$ mortality) (Fisher's test, $P = 0.027$), but was not different than the fresh-pump treatment with 99.2% survival ($n = 2$ mortalities;

Fisher's test, $P=0.064$). After 14 days of holding, survival of shad in the salt-pump, salt-air, and fresh-pump treatments was similar (99.6%, 98.4% and 99.2%, respectively; Fisher's test, $P \geq 0.216$) and significantly greater than the fresh-air treatment (79.7%; Fisher's test, $P < 0.001$; Table 3).

Handling evaluation

A significant difference was identified among the Out, In/Out, and In handling treatments (Fisher's test, $P < 0.001$; Table 3). The Out handling treatment was significantly worse than the In/Out and In handling treatments (Fisher's test, $P \leq 0.003$). Because the Out handling treatment was not a preferred method for survival, it was removed from further analyses. Dosage levels for the In/Out and In handling treatments were then compared. Comparisons of In/Out treatments (heavy and light dosage) and In handling treatments (heavy, light, control, and no dosage) failed to identify a significant difference among treatments (Fisher's test; $P=0.964$). Using these results and observational data during the handling evaluation, we chose the heavy dosage In/Out method as the best handling method for subsequent evaluations.

Tagging techniques evaluation

Two tagging dates were used in the comparison of implantation location for the tagging techniques evaluation (November 2020 and January 2021). No difference in survival of control fish from the November 2020 and January 2021 tagging dates was detected (Fisher's test; $P=0.712$), allowing control fish data to be combined.

The mixed effects Cox proportional hazards model revealed significant differences in 14-d survival between control fish and fish implanted with P1/gastric, P3/pelvic, and P4/dorsal tag type/location combinations ($P \leq 0.003$; Table 4). Differences in 14-d survival between control fish and those implanted with P2/pectoral and P5/pectoral tag type/locations were not significant ($P \geq 0.131$; Table 4). Survival rates for all groups declined over time, but the pectoral locations and control groups maintained a survival rate > 90% through day 7 post-tagging whereas the survival of all other groups was < 80% 7 days post-tagging (Fig. 6). Results from biological testing provided only a portion of the information used to determine the best prototype design.

Table 4 Results from cox proportional hazards models constructed to evaluate the effect of tag type/location on log-mortality rate

Tag type	Location	Estimate	SE	Hazard ratio	*P*-value
14-d holding					
P1	Gastric	2.466	0.616	11.779	<0.001
P2	Pectoral	1.026	0.677	2.789	0.131
P3	Pelvic	1.971	0.624	7.180	0.002
P4	Dorsal	1.897	0.629	6.666	0.003
P5	Pectoral	0.766	0.548	2.151	0.162
60-d holding					
P5	Pectoral	−0.007	0.357	0.993	0.990

Parameter estimates (the effect of tag type/location on log mortality), *SE* standard errors, hazard ratios (exponential value of the parameter estimate), and *P*-values are presented for 14- and 60-d holding experiments. A hazard ratio of 1 indicates mortality risk is equal between tagged and untagged (control) groups, hazard ratios > 1 indicate increased mortality risk in tagged fish, and hazard ratios < 1 indicate a lower risk of mortality for tagged fish. A significance level of $\alpha=0.05$ was used

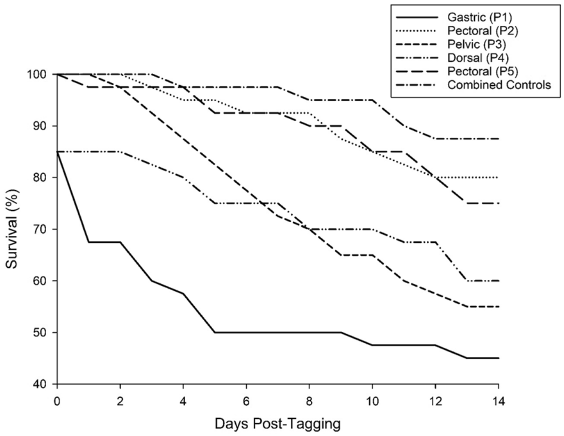


Fig. 6 Percent survival (combined survival and tag retention) for each treatment group (tagging location [prototype]) over the 14-d holding period and combined controls (November 2020 and January 2021 tagging data)

Concurrent testing of the engineering design of prototype transmitters occurred at PNNL to evaluate form factors and battery and transducer geometries. From these evaluations it was determined that the P5 prototype provided the best engineering design (unpublished data). Because P5 also performed reasonably well in the biological testing, especially considering these fish had been held at PNNL for > 4 months, it was selected as the preferred prototype for the long-term holding evaluation.

Long-term holding evaluation

The initial long-term holding evaluation was terminated when more than 75% of the shad died following the turbid water event. All of the remaining shad ($n = 73$) were used to complete a second evaluation and the 60-d results follow. The mixed effects Cox proportional hazards model revealed that the 60-d survival was similar between untagged controls and fish tagged with the P5/pectoral tag type/location combination ($P = 0.990$; Table 4).

A cursory evaluation of survival rates over time for fish held 60 days showed that survival of tagged fish was slightly lower than survival of untagged fish over the first ~ 20 days of holding (Fig. 7). However, for the rest of the study, survival of tagged shad was similar to or greater than survival of the controls (Fig. 7). Over the 60-d holding period, tagged fish grew 9.2 mm on average (median 9.5 mm; range -1 to 26 mm growth).

The GLM analysis tested whether there was a difference in fish size at the time of tagging among the P2 and P5 treatments for the tagging techniques evaluation and the long-term holding evaluation. If a difference in fish size among the treatments was found, it could bias the survival results of the evaluations. However, fish size did not have a significant effect on 14-day post-tagging survival of shad that were implanted in the pectoral location with the P2 or P5 prototypes for the tagging techniques or long-term holding evaluations ($z = 0.727$, $P = 0.467$).

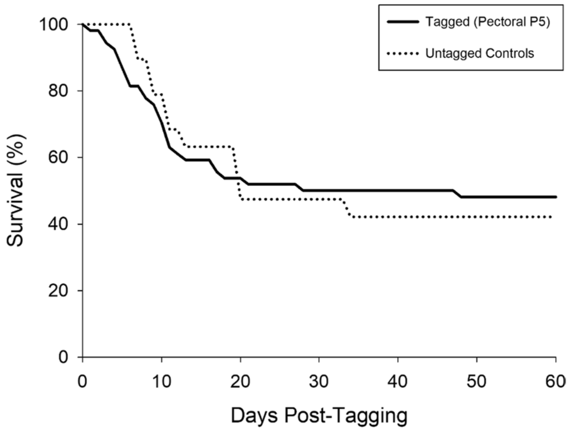


Fig. 7 Percent survival (combined survival and tag retention) for the tagged (solid line) and untagged control (dotted line) fish for the long-term holding evaluation which commenced in January 2021

Predator avoidance

No effect of transmitter implantation was observed in the survival of tagged and untagged juvenile shad exposed to predators. Of the 86 tagged juvenile shad that were exposed to predators, 23% ($n = 20$) survived without being wounded compared to 27% ($n = 23$ of 85) for the untagged group. Of the models examined for an effect on predation avoidance, the model that included only fork length provided the best fit to the survival data as determined by AIC. Though survival of tagged and untagged juvenile shad exposed to predators was negatively correlated with fork length, the model that included fork length did not provide a significantly better fit to the survival data than the intercept-only model (LRT: $\chi^2 = 3.346$; $P = 0.067$). In addition, the predictive potential of all models examined was relatively low (AUC $\leq$ 0.57; Table 5).

Discussion

This two-year study evaluated the effects of transportation, holding, handling, and tagging on juvenile American shad. Modifications made to our previous collection and transportation methods allowed us to hold untagged American shad at the ARL for over 4 months in freshwater. However, when shad were tagged with an acoustic transmitter, we found the use of saltwater (7.5 ppt) during tagging and 1 d post-recovery to be essential for keeping the fish alive. The preferred tagging method involved using a heavy dose of anesthesia (120 ppm MS-222) with an In/Out handling method (in-water transfer to a pre-tag source bucket/out-of-water transfers for anesthesia, measuring, and tagging) to implant the transmitter into a pectoral incision with no suture. The P5 prototype was the ideal design due to the form factor and geometries of the battery and transducer. When these handling, holding, and tagging methods were used, there was

Table 5 Results of generalized linear models fit to the binomial predator exposure survival data of tagged and untagged juvenile shad as a function of intercept (β_0), fork length ($\beta_1 FL$), tagged or untagged treatment ($\beta_2 T$), and an interaction of fork length and treatment ($\beta_3 FL \bullet T$). β_2 coefficients are shown for tagged fish. Standard errors of variable coefficients are shown in parentheses

Model	β_0	β_1	β_2	β_3	AIC	AUC
β_0	− 1.091 (0.176)				194.89	0.50
$\beta_0 + \beta_1 FL$	2.228 (1.837)	− 0.042 (0.023)			193.52	0.57
$\beta_0 + \beta_2 T$	− 0.992 (0.244)		− 0.202 (0.353)		196.54	0.53
$\beta_0 + \beta_1 FL + \beta_2 T$	2.318 (1.851)	− 0.042 (0.023)	− 0.193 (0.357)		195.37	0.57
$\beta_0 + \beta_1 FL + \beta_2 T + \beta_3 FL \bullet T$	2.517 (2.692)	− 0.045 (0.034)	− 0.568 (3.693)	0.005 (0.047)	197.22	0.57

AIC Akaike information criterion, *AUC* Area under the curve

no difference in long-term survival or predator avoidance of tagged shad compared to untagged controls.

Transport evaluation

Our results indicate that reproducible high rates of survival for transporting juvenile shad can be obtained by implementing the following protocols: using a soft mesh net and water-to-water transfer during collection, using a circular tank with an integrated water pump to circulate the flow at a constant water velocity, using a water salinity of approximately 7.5 ppt, providing a mechanism to reduce sloshing during transport (i.e., foam pad on water surface), and water-to-water transfer for unloading fish to holding tanks. Other studies have suggested that using a pump intermittently throughout transport (Sykes 1950) or keeping tank velocities below 0.3 m s^{-1} and reducing the use of agitator aerators to provide minimal directional flow (Raquel et al. 1989) may aid in transport survival. Our study used two different methods of providing constant directional flow to the circular transport tank and found no difference between the two methods.

Although the directional flow did not affect survival, the methods employed while sorting and collecting shad at the McNary Dam bypass facility may have affected survival. Initially, fish sorting and collection had not been considered a potential stressor and were considered independent of the transportation. Upon observation of the collection process and further assessment of the survival results from the first transportation event, it was clear that changes could be made to improve that process as well. Modifications to the collection process, such as minimizing the time fish were held in a net by alternatively holding them in saltwater until they could be placed in the saltwater transport tank, resulted in 99% survival in the first 24 h from collection. This survival rate is nearly a 25% increase in survival compared to the first transportation event and approximately a 50% increase from previous years of collecting juvenile shad from McNary Dam (Deters et al., unpublished data).

For all shad transportations over this two-year study, mortality was highest during the first 24 h after departing McNary Dam, ranging from 1.4 to 22% in year one and 10–14% in year two, with an overall average of 11.7%. In a study comparing the acute handling and confinement stress response of shad held in freshwater to those held in saltwater, saltwater-held fish were able to return to basal cortisol levels within 24 h after the stressor, whereas freshwater-held fish were not and the plasma cortisol levels in the freshwater fish were three times higher than the saltwater fish (Shrimpton et al. 2001). Significant improvements were made in transportation survival at PNNL from 2018 to 2020; however, further investigation into the possible benefits of a 24 h saltwater hold following the stress of transportation is warranted.

Holding

Our holding evaluation compared the two-week survival of shad held in approximately 8 ppt saltwater compared to freshwater. Fish in the fresh-air treatment, which included air-lift transport and freshwater hold, had the lowest survival (80%) compared to the other three treatments (> 98% survival for each). The higher mortality in the fresh-air tank may have been the result of disease that spread throughout the tank. On day seven, several mortalities from the fresh-air tank exhibited signs of tail-rot. When mortality in this tank peaked on day 13, fungus was observed on some of the mortalities. This suggests that when challenged with disease exposure, the fish in the freshwater (fresh-air) treatment were not able to overcome that stress compared to fish held in saltwater. This aligns with other observations wherein shad held in freshwater experienced higher mortality after a stress event than shad held in saltwater (Shrimpton et al. 2001). Because of the potential confounding results of the disease, we compared survival after one week. However, mortality had already increased quite rapidly on day six and the fresh-air treatment still had the lowest survival compared to the other three treatments.

Aside from the holding evaluation, all American shad brought into the lab were held on freshwater for the entire study period (September–January). Contrary to a previous finding that juvenile American shad could not be held in freshwater in a laboratory setting beyond December regardless of water temperature (Zydlewski and McCormick 1997), fish transported in September of 2020 and September 2021 for this study were held in ambient freshwater (with the exception of the post-tagging saltwater hold) through the end of January 2021 in year one (minimum 9.1 °C water temperature) and through mid-March 2022 in year two (minimum 14.9 °C). The use of formalin treatments during times of increasing mortality was an effective means of controlling outbreaks of disease and improving fish health and survival. However, formalin was not used during active evaluations to avoid confounding results.

Handling

The handling evaluation, which compared ten different handling scenarios, had lower than expected survival across treatments, including some of the treatments that experienced relatively less handling (e.g., control/In). One possible explanation for the lower overall survival is that these fish came from the saltwater holding tanks used in the 14-day holding evaluation. The study plan was to use the freshwater tanks as the source for the handling evaluation, but that was not possible due to the high mortality and disease in one of the freshwater tanks. Instead, the test fish went from a 14-day saltwater environment to a freshwater handling event and were then held in a freshwater environment for another 14 d. It is possible that fish were unable to osmoregulate when transferred from saltwater to freshwater, particularly after a stressful event. For example, in an osmoregulatory challenge, researchers observed 50% mortality after 24 h and significant declines in plasma sodium, chloride, and osmolality when juvenile shad held in 12 ppt saltwater were transferred to 0 ppt freshwater (Zydlewski and McCormick 1997). However, no mortality occurred and no changes in plasma sodium, chloride, or osmolality were observed in shad transferred from 12 to 24 ppt saltwater. Considering the benefits of holding fish in saltwater observed in this study, fish in the handling evaluation may have had higher survival had it been conducted in saltwater and if the fish had been given time to recover in saltwater as well.

The use of saltwater has produced noticeable benefits to the transportation and tagging efforts at PNNL since 2018. However, these evaluations were completed over an extended time and they did not test the effects of several days of saltwater holding (i.e., collection, transportation, tagging, post-tagging hold) in a condensed timeframe. Rather, the collection and transportation events occurred one to four months prior to the tagging events. It is unknown if negative effects may be associated with multiple highly stressful events in a short timeframe. More research is needed to understand the effect of multiple days of saltwater holding prior to releasing shad into a freshwater river environment for a dam passage evaluation.

Tagging techniques

Gastric tagging is the most common method for implanting transmitters into adult clupeids (Bunch et al. 2023; Castro-Santos and Vono 2013; Moser et al. 2000; Smith et al. 2009; Tetard et al. 2016). Gastric techniques typically utilize laboratory tools or repurposed objects (e.g., plastic tubing) to hold the transmitter while another object is used to push the tag carefully into the stomach (Bunch et al. 2023; Castro-Santos and Vono; Smith et al. 2009). Some gastric tagging studies use anesthesia to subdue the fish (Acolas et al. 2004), while most do not (Castro-Santos and Vono 2013; Eakin 2017; Grote et al. 2014;

Moser et al. 2000; Smith et al. 2009; Tetard et al. 2016). In this study, scale loss was so great even with the use of anesthesia and saltwater holding that we did not attempt a no-anesthesia method for gastric tagging juvenile shad.

The gastric technique was the only tagging method that resulted in tag loss in our study. After 1 d of holding for the tagging techniques evaluation, 25% of the gastric-tagged fish had expelled their transmitters. This differed greatly from the results in the initial prototype evaluation, which tested smaller sample sizes. Of the fish gastric-tagged with the P1 design, only one of 10 fish lost a transmitter, which occurred on day one. Regurgitation of tags has occurred in other gastric tagging studies and the use of rubber bands has helped to reduce regurgitation (Keefer et al. 2004). However, Bunch et al. (2023) observed no tag loss in adult shad gastric-tagged with cylindrical transmitters, so it is possible that tag retention rates may differ with fish size or life stage. We recommend further evaluation of the tag loss associated with the gastric method in juvenile shad, as this could be a viable option for a non-invasive tagging method; the mortality rates for the gastric and pectoral locations were comparable in this study.

External attachment of transmitters is another common method of tagging adult clupeids (Bolland et al. 2019; Breine et al. 2017; Castro-Santos and Haro 2015). External attachment has been used in short-term entrainment (Mathur et al. 2018) and turbine passage studies (Heisey et al. 1992) and in lab studies conducted to evaluate the effects of tagging on Atlantic herring (*Clupea harengus*; Stobo et al. 1992) and American shad (Knight et al. 1977). The field studies were successful in part because of the short-term evaluation period. However, even with the short-term nature of the turbine study, survival was improved when fish were held in saltwater (Heisey et al. 1992). The 24-h mortality for controls was 56% when held in freshwater compared to 0% when held in 5 ppt saltwater prior to tagging. In addition, studies testing externally tagged fish have suggested a relatively large minimum size guideline of 170 mm for herring (Stobo et al. 1992) or have performed the tests where outmigrants are larger (Mathur et al. 2018). Mean length of Susquehanna River outmigrants is approximately 125 mm compared to the approximately 90 mm American shad migrants from the Connecticut River (Mathur et al. 2018). The external tag used in our study was designed to be neutrally buoyant (i.e., tag burden of 0%), but the dorsal attachment method had lower survival than controls in our evaluation.

Surgical techniques for implanting transmitters in clupeids appear to be the least common technique, but the results have been favorable in the few studies that have evaluated it. The first successful juvenile shad tagging study used a surgical technique with a pelvic incision, but presumably without the use of anesthesia to implant the tag (Hollis 1948). Following implantation, shad were held in Ringer's solution (an isotonic solution made up of several salts) for approximately two weeks. Survival with tag retention was around 56%. Recently, researchers in England used a systematic approach to test novel methods of tagging adult twait shad (*Alosa fallax*) with acoustic transmitters (Bolland et al. 2019). Once they had upstream detections of shad that had been anesthetized, tagged with an external transmitter, and released, they advanced to surgical implantation of the transmitter into the peritoneal cavity. Additionally, Gahagan and Bailey (2020) surgically implanted acoustic transmitters into emigrating American shad and succeeded in tracking their return to the river to spawn the following year. Although few studies have evaluated the use of surgical incisions for tag implantation in alosines, the results from these studies and the present study suggest it is a valid option.

Long-term holding

We planned to test a larger sample size and larger size range of American shad in both the tagged and control groups for this evaluation. However, conditions out of our control, such as fewer fish at collection, larger overall size of fish collected, and fish mortality due to the turbid water event prevented a more robust evaluation. However, the results may still be very useful to researchers using acoustic telemetry to study juvenile American shad and other small, sensitive species.

Results from the Cox proportional hazards model indicated juvenile shad implanted with the P5 design in the pectoral region experienced similar survival (48%) to untagged controls (42%) over the course of the 60 d holding study. In comparison, hatchery-reared adult Delta smelt (*Hypomesus transpacificus*; > 70 mm FL) surgically implanted with a 0.22 g acoustic tag were held for 28 d and survived at a rate of 60% compared to 100% survival for the non-tagged anesthesia control (Wilder et al. 2016). In the same study, Delta smelt injected with a 0.03 g passive integrated transponder (PIT) tag survived at a rate of 95%. Shad tested during our 60-day holding evaluation were of similar weight to the Delta smelt in that study. However, the tag in this study was more comparable in size (maximum tag burden 1.5%) to the PIT tag (average tag burden 0.8%) than the acoustic tag (4–8%). Our tagged fish had a lower survival rate (50.0% on day 28) than the Delta smelt PIT group, but our control fish experienced similar survival (47% on day 28) to the tagged shad, possibly indicating the extreme sensitivity of juvenile American shad to handling.

The authors postulated that for Delta smelt to survive at a rate of 90% or more, an acoustic transmitter would need to weigh no more than 2% of the fish's body weight (Wilder et al. 2016). The transmitter developed for this study is light enough to meet that requirement. The dummy tags tested in this evaluation weighed 0.058 g, but the weight of the transmitter with functioning components and battery is expected to be 0.050 g. The development of this new transmitter will allow the evaluation of acoustic tagged adult Delta smelt with a tag burden of approximately 1.5%.

Predator avoidance

We found that tagged juvenile American shad did not exhibit an impaired ability to avoid predators when compared to untagged control fish. We were unable to find any other studies of juvenile shad that used predator avoidance as a metric to evaluate tagging effects in the literature. One study using juvenile Chinook salmon found no differences in the proportion of fish preyed upon by rainbow trout (*Oncorhynchus mykiss*) when implanted with an acoustic transmitter; however, these fish were larger than the shad in our study (on average 140 mm FL with a tag burden up to 6.7%; Anglea et al. 2004). In a similar study with smaller fish, Walker et al. (2016) found no effect on predator avoidance in Chinook salmon implanted with an injectable acoustic transmitter (85–95 mm, tag burden 3.0–5.3%). Similarly, there were no differences for Chinook salmon (98–135 mm FL) tagged with a neutrally buoyant external transmitter when compared to untagged controls (Janak et al. 2012). Our results indicate that American shad with a tag burden up to 4.1% showed no increased risk of predation in a controlled laboratory setting. Although future studies with larger sample sizes would help validate our results, this study suggests that tagged shad will not face increased predation rates compared to their non-tagged counterparts in field studies that implement this tagging technique.

Conclusions

In this two-year laboratory study, we evaluated the collection, transportation, holding, handling, tagging, and predator avoidance of juvenile American shad. After improving collection methods, 24 h transportation survival increased to 99%. We identified a successful handling method for tagging juvenile shad (50–105 mm) with an acoustic transmitter using a pectoral incision with no suture. After 30 days, which is the expected life of the acoustic transmitter, survival in the 60-d holding evaluation was similar for tagged fish (50%) and the untagged controls (47%) We observed no difference in survival between tagged shad and untagged controls for the predator avoidance evaluation.

This study has encouraging results for broadening the application of telemetry and improving conservation efforts for small, sensitive, and threatened species of fish such as American shad, Eulachon (*Thaleichthys pacificus*), and Delta smelt. The collection, transport, handling, and tagging techniques evaluated in this study can be used as a template for future field evaluations using acoustic telemetry to monitor American shad migration to aid in determining entrainment at hydropower facilities and survival rates through river reaches. In addition, these techniques can be easily adapted for implanting shad with PIT tags, which can provide additional long-term monitoring of shad throughout their life history.

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Data availability Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare there are no competing interests.

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