Elsevier Editorial System(tm) for Fisheries Research
Manuscript Draft

Manuscript Number: FISH8593R1

Title: Refinement of acoustic-tagging protocol for twaite shad Alosa fallax (Lacépède), a species sensitive to handling and sedation

Article Type: Short Communication

Keywords: Anadromous; Animal welfare; Iteroparous; Regulated procedure; Surgical implantation; Telemetry

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Manuscript Region of Origin: UNITED KINGDOM

Abstract: Telemetry investigations to gather essential information about fish migrations are strongly reliant on the behaviour, energetics, condition and survival of the animals being unaltered by the tagging procedure. Twaite shad (Alosa fallax Lacépède; 'shad') is a threatened clupeid fish for which there is a considerable knowledge gap on their anadromous movements. They are also reported to be sensitive to handling and anaesthesia, resulting in practical difficulties in tag implantation; previous investigations externally attached tags without sedation. The aim of this study was to incrementally refine the acoustic-tagging protocol for shad via application of a previously un-tried anaesthetic (i.e. tricaine methanesulphonate (MS-222)) and by surgical implantation of the tag in the peritoneal cavity. All captured shad (n = 25) survived handling, anaesthesia and tagging, and were detected moving upstream after release. Surgically implantation (n = 5) was significantly faster than externally mounting the tag (n = 20) and time to recover was similar. Total upstream movement, total movement, residence time in receiver array and speed of upstream movement were statistically similar for externally and internally tagged fish. Post-spawning, a large proportion (68 %) of tagged fish returned to the estuary, downstream of the receiver array. Internal tagging under anaesthesia is recommended for studying anadromous movements of shad, given welfare benefits during surgery and once at liberty, thus increasing the likelihood of tagged fish performing natural behaviours. Further, implantation of tags programmed to last many years enables multiple spawning migrations by the same individuals to be studied, which would lead to substantial advances in ecological knowledge and potentially reduce the total number of tagged fish.

Refinement of acoustic-tagging protocol for twaite shad *Alosa fallax* (Lacépède), a species sensitive to handling and sedation

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ABSTRACT

Telemetry investigations to gather essential information about fish migrations are reliant on the behaviour, condition and survival of the animals being unaltered by the tagging procedure. Twaite shad (*Alosa fallax* Lacépède; 'shad') is a threatened clupeid fish for which there is a considerable knowledge gap on their anadromous movements. They are also reported to be sensitive to handling and anaesthesia, resulting in practical difficulties in tag implantation; previous investigations externally attached tags without sedation. The aim of this study was to incrementally refine the acoustic-tagging protocol for shad *via* application of a previously un-tried anaesthetic (i.e. tricaine methanesulphonate (MS-222)) and by surgical implantation of the tag in the peritoneal cavity. All captured shad (n = 25) survived handling, anaesthesia and tagging, and were detected moving upstream after release. Surgically implantation (n = 5) was significantly faster than externally mounting the tag (n = 20) and time to recover was similar. Total upstream movement, total movement, residence time in receiver array and speed of upstream movement were statistically similar for externally and internally tagged fish. Post-spawning, a large proportion (68 %) of tagged fish returned to the estuary, downstream of the receiver array. Internal tagging under anaesthesia is recommended for studying anadromous movements of shad, given welfare benefits during surgery and once at liberty, thus increasing the likelihood of tagged fish performing natural behaviours. Further, implantation of tags programmed to last many years enables multiple spawning migrations by the same individuals to be studied, which would

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Keywords

Anadromous; Animal welfare; Iteroparous; Regulated procedure; Surgical implantation; Telemetry

1. Introduction

Fish telemetry investigations are routinely performed to gather essential information about migrations, habitat use, predator–prey interactions and responses to anthropogenic impacts, to help protect species and the environments they inhabit (Hussey et al., 2015). Such studies are reliant on the behaviour, condition and survival of the animals being unaltered by the tagging procedure (Cooke et al., 2013). This has resulted in a considerable amount of work to identify maximum tag burden, optimal tag implantation location and most appropriate methods of anaesthesia (Broadhurst et al., 2009; Ross & Ross, 2009). There have been considerable refinements in internal tagging procedures, with tags often retained for the lifetime of the fish with minimal long-term impact (Jepsen et al., 2002; Bridger and Booth, 2003; Cooke et al., 2011). External tag attachment remains important in some studies and species, including those considered to be sensitive to handling (Jepsen et al., 2015; Johnson et al., 2015). However, tags can become fouled, increase drag during swimming, cause irritation and harm as the fish grow, potentially impairing individual behaviour and increasing mortality risk (Mulcahy, 2003; Cooke et al., 2013; Jepsen et al., 2015).

Twaite shad *Alosa fallax* (Lacépède) (‘shad’ hereafter) is an anadromous clupeid fish species that was once abundant and widespread across Europe (Aprahamian et al., 2003). Their populations have, however, declined considerably in the last century. Causal factors relate primarily to anthropogenic disturbances, especially the construction of weirs in the lower reaches of rivers that reduce access to spawning areas (Jolly et al., 2012). The species is listed on Appendix III of the Bern Convention and Annexes II and V of the EU Habitats Directive. Despite their conservation importance, their anadromous spawning migration remains under-studied primarily due to difficulties tagging shad, a species reported to adversely react to handling and sedation (with 2-phenoxyethanol) that results in high mortality rates (Rooney and King, 2014; Breine et al., 2017). To overcome these challenges, recent investigations have externally mounted acoustic tags without sedation because it is less invasive and thought to be quicker than surgical implantation (Rooney and King, 2014; Breine et al., 2017). Although these studies were successful, Breine et al. (2017) recommended further research on the effects of anaesthesia, handling and tagging on shad.

The aim of this study was to refine the acoustic-tagging protocol for shad, giving due consideration to their sensitivity to handling and sedation, to provide short-term welfare benefits during surgery and long-term welfare benefits while at liberty, thus enabling expression of natural behaviours. Objectives were to: (1) refine the external tag attachment protocol of Breine et al. (2017) *via* application of previously un-tried anaesthetic (i.e. tricaine methanesulphonate (MS-222)); (2) further refine the procedure by surgically implanting the tag within the peritoneal cavity; and (3) quantify the impacts of the tagging methods through comparison of shad movement. As shad are iteroparous and, potentially, philopatric (King and Roche, 2008), implantation of tags programmed to last many years enables multiple spawning migrations by the same individuals to be studied, which would lead to substantial advances in ecological knowledge.

2. Methods

2.1. Fish capture and iterative tagging process

The refinement of the shad tagging protocol was completed during the 2017 shad spawning migration in the River Severn, Western England (Fig. 1). Twenty-five shad were captured from two locations, downstream of Maisemore (n = 8) and Upper Lode weirs (n = 17), with 23 captured by angling (small lure with single barbless hook) and two with a seine net (30-m long, 2-m deep and 10-mm mesh) (Table 1). Tagging was an iterative process involving small batches of fish to minimise the number of fish with compromised welfare if tagging was unsuccessful and to enable refinements between batches. Thus, the initial 3 captured fish were externally tagged under general anaesthesia (batch 1), with tagging only recommencing once a receiver 14.8-km upstream of the release location revealed the fish had recovered sufficiently to continue their upstream movement. The decision to commence surgically implanting tags in the body cavity (batch 4) was only taken after a further 11 shad had been successfully tagged externally (batch 2 and 3). The final six fish (batch 5) were tagged externally because there was no opportunity to establish if the internally tagged fish (batch 4) had been detected on the receiver upstream of the release location.

Table 1. Capture date, sample size and capture, release (DS = downstream, US = upstream) and tag locations of twaite shad tagged in five batches on the River Severn.

Batch	Date	*n*	Capture location	Release location	Tag location
1	11/5/17	3	DS Maisemore Weir	US Maisemore Weir	External
2	17/5/17	5	DS Upper Lode Weir	US Upper Lode Weir	External
3	17/5/17	6	DS Upper Lode Weir	DS Upper Lode Weir	External
4	22/5/17	5	DS Maisemore Weir	US Maisemore Weir	Internal
5	31/5/17	6	DS Upper Lode Weir	DS Upper Lode Weir	External

2.2. External and internal tagging procedures

Prior to tagging, acoustic tags (20-mm long x 7-mm diameter (V7), 1.6-g weight in air and 29-mm long x 9-mm diameter (V9), 4.7-g weight in air; www.vemco.com) were activated and tested with a hand-held detector to verify they were transmitting; weight in air did not exceed 2% of fish mass. Following capture, fish were briefly held in water filled containers (100 L) prior to their general anaesthesia (MS-222; 0.4-g per 10-L of water). All fish were inspected for signs of pre-existing injury and disease; no captured fish were excluded from tagging. Whilst being sedated, the fish were measured (fork length, nearest mm; mean ± S.D.: 354 ± 37 mm, range = 302–420 mm), and scale sample and a fin biopsy taken (for use in complementary studies). The influence of the anaesthetic was visually assessed using body, opercula and eye movements, with fish only removed following their lack of a response to touch, loss of ability to balance and the cessation of pectoral fin and eye movements.

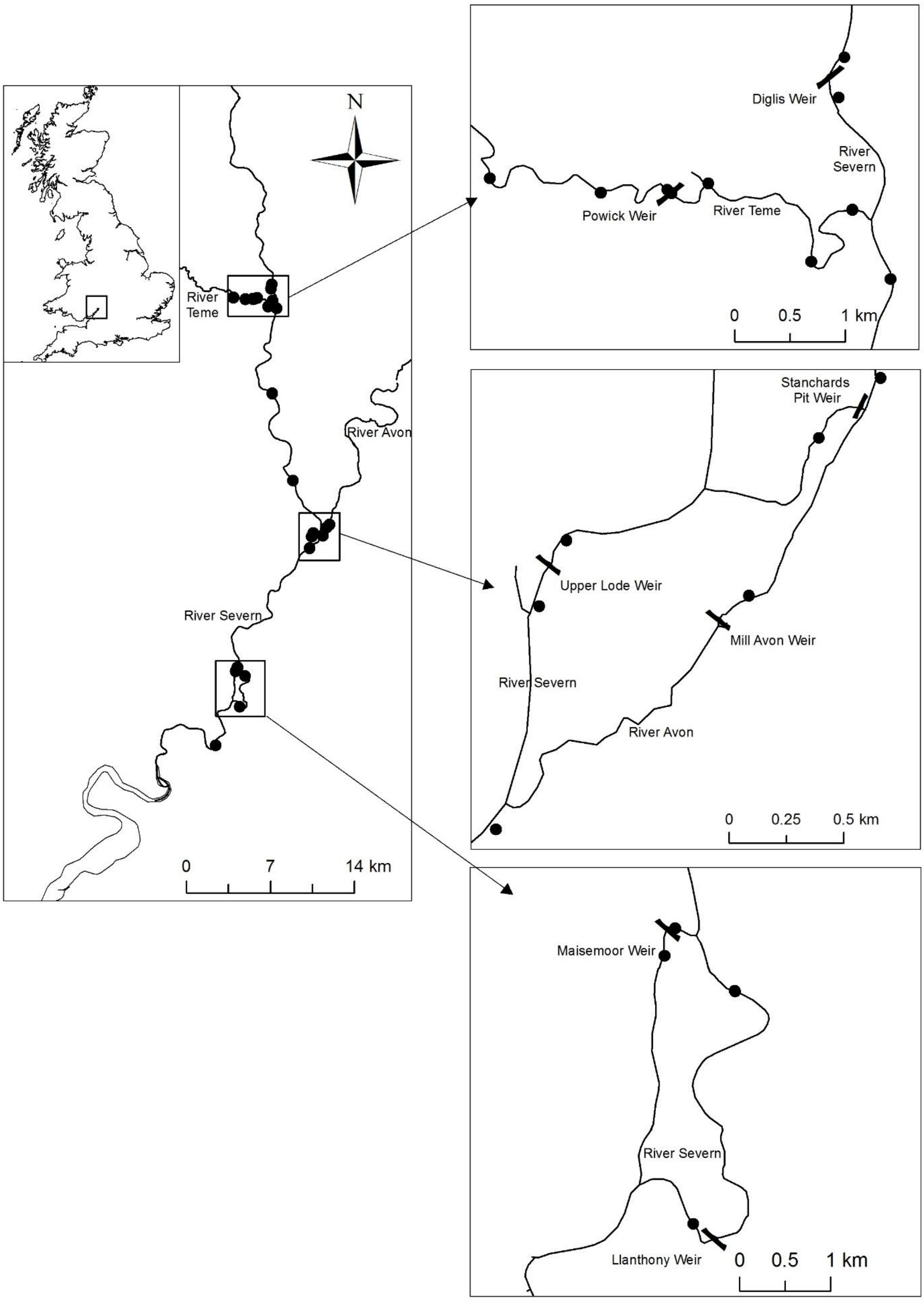

Figure 1. A map of acoustic receiver locations (black dots) in the River Severn catchment, including impediments to fish migration (black lines). Maisemore and Llanthony weirs represent the tidal limit, and Maisemore and Upper Lode weirs were capture locations.

Externally mounted tags were attached using surgical thread (Ethilon) passed through the dorsal musculature using hollow needles and held in place using a rubber plate and aluminium sleeves (as per Breine et al., 2017). Surgically implanted tags were disinfected with providone-iodine and rinsed with saline solution before being implanted into the body cavity through a ventro-lateral incision made with a scalpel, anterior to the muscle bed of the pelvic fins. The incision was closed with an absorbable monofilament suture. Fish were held in a clean V-shaped foam support and their eyes were covered with a damp cloth during surgery. All fish were treated in compliance with the UK ASPA (1986) Home Office licence number PPL 60/4400.

After surgery, fish were transferred to a damp sling for weighing (to 25 g; mean ± S.D. = 547 ± 173 g, range = 300–850 g) and then returned to the river, being held whilst they orientated towards the flow and were only released when they had regained balance, body reflexes and swimming ability. This was considered preferable to holding fish in tanks with water circulation and aeration, as shad have been recorded to die during transportation and at fish farms (Clough et al., 2004). Fish were released upstream of Maisemore Weir (n = 8), downstream of Upper Lode Weir (n = 12) and upstream of Upper Lode Weir (n = 5) as part of the wider investigation (Table 1). Catchment-wide migration was examined using 23 strategically located acoustic receivers (Vemco; www.vemco.com) (Fig. 1); no fish were detected on the most upstream receivers.

2.3. Data analysis

Time taken for anaesthesia, surgery and recovery when externally and internally tagging shad was compared using t-tests (non-normal data (Shapiro test) were log-transformed). It was not possible to recapture tagged shad to assess general health and condition, external tag fouling or healing of incisions for internally implanted tags. Instead, movements of fish in the river were used as evidence that the fish had recovered from handling, anaesthesia and surgery. Specifically, the amount of upstream movement (i.e. sum of all upstream movements), total movement (i.e. sum of all up and downstream movements), and residence time in the receiver array (i.e. number of days from release to first detection on last receiver) were calculated for each fish. In addition, the speed of upstream movement between receivers was calculated (distance between receivers / last detection on upstream receiver – first detection on downstream receiver). The movements of fish in batches 1 and 4, captured and released at the same location but with external and internal tag attachment, were compared using t-tests (non-normal data (Shapiro test) were log-transformed) to quantify impacts of the tagging methodology. Both movement and speed metrics represent minimum estimates, as they are measured at the resolution of receiver separation, thus back and forth movements between receiver detection area are undetected. The fates of individual fish were broadly separated into those that returned to the estuary and those that were assumed to have died in the river, though the latter could not be separated from tag failure or loss, and the potential cause of death could not be determined (e.g. tagging induced, natural predation event, tagging-induced predation event or natural mortality after spawning). Data analysis was performed primarily in Microsoft Excel and statistical comparisons performed using R statistical software (version 3.4.3, R Core Team 2017), with movement speed analysis in the V-Track package (Campbell et al., 2012).

3. Results

All 25 fish caught during the investigation survived capture, handling, sedation and tagging, and were assessed as being in satisfactory condition prior to be returned to the river. The time taken for anaesthesia was similar (t = -0.054171, d.f. = 5.5144, P = 0.959) whereas internal implantation was significantly faster than external attachment (t-test on logged data; t = -88.36, d.f. = 32.372, P <0.001), both usually within four minutes (Table 2). The mean time to recover was also similar (t-test on logged data; t = -1.9709, d.f. = 7.8191, P = 0.085), and the longest recovery did not exceed six minutes for either treatment group (Table 2).

Table 2. Time (seconds; mean ± 95% C.I. (min.–max.)) taken for anaesthesia, surgery and recovery when externally and internally tagging shad with acoustic tags.

Procedure stage	External (*n* = 20)	Internal (*n* = 5)
Anaesthesia	112 ± 12 (60–182)	113 ± 28 (70–150)
Surgery	113 ± 10 (83–179)	117 ± 12 (104–136)
Recovery	149 ± 28 (85–356)	196 ± 54 (140–301)

All shad were detected moving upstream in fresh water, i.e. against the flow. Of all the batches, the first batch of fish (external tag) had the greatest mean upstream movement (61.1 ± 51.7 km) and mean total movement (122.9 ± 95.2 km), whereas the fourth batch (internal tag) spent the longest mean time in the river (21.4 ± 8.8 days) and fastest mean speed of upstream movement (1.10 ± 0.32 m/s) (Table 3). Fish in batches 1 and 4 were captured and released at the same location with external and internal tags, respectively, and had similar upstream movements (*t*-test on logged data; t = 0.095988, d.f. = 3.7202, P = 0.926), total movements (*t*-test on logged data; t = 0.31356, d.f. = 4.3419, P = 0.768), times in the river (*t*-test; t = -0.61932, d.f. = 5.5427, P = 0.560) and speed of upstream movements (*t*-test; t = 2.1894, d.f. = 6, P = 0.0711) (Table 3). The individual fish with the greatest upstream (138.0 km) and total movements (281.4 km), and longest time in the river (29.8 days) had an internal tag, whereas the fastest upstream movements (1.79 m/s) was by a fish that had an external tag.

Table 3. Mean ± 95% C.I. (min.–max.) upstream movement (km), total movement (km), residence time in the receiver array (days) and speed of upstream movement (m/s) for shad tagged in five batches on the River Severn.

Batch	Upstream movement (km)	Total movement (km)	Time in river (days)	Speed of upstream movements (m/s)
1	61.1 ± 51.7	122.9 ± 95.2	18.3 ± 4.4	0.60 ± 0.19
	(27.7–113.1)	(60.4–218.5)	(13.9–21.2)	(0.50–0.80)
2	16.4 ± 11.4	50.8 ± 26.5	12.8 ± 5.0	0.54 ± 0.14
	(4.0–37.7)	(19.0–96.5)	(6.6–23.3)	(0.30–0.73)
3	14.4 ± 11.7	46.2 ± 28.7	8.4 ± 4.5	0.51 ± 0.17
	(1.0–33.9)	(5.7–91.4)	(0.2–16.2)	(0.31–0.77)
4	58.0 ± 39.6	112.1 ± 83.6	21.4 ± 8.8	1.10 ± 0.32
	(30.7–138.0)	(51.0–281.4)	(9.3–29.8)	(0.72–1.52)
5	15.5 ± 11.6	49.0 ± 16.4	8.9 ± 5.7	1.09 ± 0.38

(2.0–38.6)	(28.3–73.7)	(1.5–19.1)	(0.55–1.79)

Seventeen (68%) of the tagged shad performed a downstream migration to the estuary between 25 May and 21 June 2017, 14.7 ± 3.9 days after tagging. Eight fish were assumed to have died in the river (though tag failure or loss could not be ruled out) but were tracked for a similar amount of time, i.e. 10.6 ± 8.2 days. The one exception (external tag) was last detected 5 h after release, 5.7 km upstream of its release location. Four fish (external = 2 and internal = 2) were last detected in the vicinity of a suspected spawning location 9–27 days after release, three of which moved downstream after release and subsequently returned to fresh water. Three fish (external = 2 and internal = 1) were last detected moving downstream 5, 7 and 12 days after release, each having moved a minimum of 18.7, 4.0 and 36.3 km, respectively, in an upstream direction while in fresh water.

4. Discussion

During this investigation, twaite shad, a threatened anadromous fish species that is sensitive to handling and sedation, were successfully anaesthetised which enabled tags to be surgically implanted into the peritoneal cavity. These findings are contrary to Rooney and King (2014) who reported mortality of shad anaesthetised with 2-phenoxyethanol and represents a substantial refinement of an accepted tagging protocol (*cf*. Breine et al., 2017). The novel and successful use of MS-222 for shad might be reflective of high variability in species-specific responses to different anaesthetics (e.g. Readman et al., 2017). These refinements have important welfare, ethical and methodological implications for future shad tracking studies.

Twaite shad are anadromous and iteroparous. In this study, a large proportion of the tagged fish (68%) migrated downstream to the estuary after undertaking substantial movements upstream and spent an appreciable amount of time in fresh water. This suggested that tagging had little or no impact on their behaviour and that these fish evaded predators (e.g. pike *Esox lucius* L., zander *Sander lucioperca* (L.), otter *Lutra lutra* (L.) and cormorant *Phalacrocorax carbo* L.) and survived spawning. The assumed mortality of individuals that did not return to the estuary (though tag failure or loss could not be ruled out) was considered a result of either natural predation or post-spawning mortality, rather than a direct consequence of being tagged. This is because they performed substantial upstream movements, entered the estuary and returned to fresh water, were last detected at a suspected spawning location and/or residence time was similar to fish that returned to the estuary.

A commonly cited advantage of external tagging over surgical implantation is that attachment can be faster (Jepsen et al., 2015; Breine et al., 2017), but internal implantation was significantly faster than external attachment in this investigation. Although there was no evidence of detrimental impacts of externally mounting tags they may have reduced swimming performance through drag or disequilibrium. There are many other long-term benefits of internal implantation to individual fish post-release, including improved tag retention, reduced tissue damage, zero risk of biofouling and zero tag visibility to predators (Cooke et al, 2013; Jepsen et al., 2015). Surgically implanting long-lived tags will also provide substantial advances in ecological knowledge of iteroparous shad by enabling multiple annual spawning migrations of the same individual to be studied. Consequently, the number of fish that need to be tagged could also be reduced, thereby complying with the reduction principle of animal research (Metcalfe and Craig 2011). These refinements should be transferable to other fishes considered sensitive to handling and sedation, and should lead to further refinements in tagging procedures during biotelemetry research.

Acknowledgments

Funding was received from the Unlocking the Severn for LIFE Programme, a partnership between the Canal & River Trust, Severn Rivers Trust, Environment Agency and Natural England through funding from the EU LIFE Nature Programme (LIFE15/NAT/UK/000219) and Heritage Lottery Fund (HLF-15-04573). The authors would like to thank all landowners for access and Tewkesbury Popular Angling Association for assisting with fish capture.

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