Chapter 10

Addendum

10.1 Successful external acoustic tagging of twaite shad *Alosa fallax* (Lacépède 1803)

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10.1.1 Abstract

Although twaite shad declined substantially in many European rivers, its numbers increased since 2007 in the Belgian Zeeschelde. Since twaite shad is a

species of conservation concern, further knowledge on its migration and reproductive behaviour is needed and acoustic telemetry would be a relevant tool to assess these behaviours. Shads are very sensitive fish showing adverse reactions to handling and anesthesia, specifically twaite shad. Therefore, this species is rather unsuitable for internal implantation of electronic tags, such as acoustic, radio and data storage tags. Tests are needed to assess the impact of external tagging on twaite shad survival. Here we describe a fish friendly attachment procedure to externally tag the fish. The procedure is quick and may limit additional drag force on swimming as the tags are firmly attached to the body by a rubber plate. This procedure was developed in Belgium in spring 2015 to tag eight shads in the Zeeschelde. Five of these shads showed a migration pattern that generally corresponded with spawning activities observed visually in the river.

10.1.2 Introduction

Twaite shad is an iteroparous, anadromous clupeid occurring along the European coast from Morocco to the Baltic Sea, throughout the Mediterranean Sea and along the Northeastern Atlantic Coast (Aprahamian et al., 2003a; Maitland and Lyle, 2005). It is a marine pelagic fish species, but migrates during spring into the middle and upper reaches of the river to spawn (Maes et al., 2008).

Since the early nineties, a strong decline in twaite shad populations has been observed due to anthropogenic influences, such as water pollution, modification of river habitat and hydrology, and overfishing (Assis, 1990; Bervoets et al., 1990; Doherty et al., 2004). Following its decline, the species is classified as vulnerable and listed under the International Union for the Conservation of Nature (IUCN) World Red Data Book (IUCN, 2015), included in Appendix III of the Bern Convention (CE, 1979) and Annexes II and V of the EC Habitats Directive (Aprahamian et al., 2003b). Despite a recent population increase in the Rivers Seine, Rhone, Ebro, Schelde, Elbe and Curonian Lagoon (Belliard et al., 2009; Lebel et al., 2001; López et al., 2007; Maes et al., 2008; Magath and Thiel, 2013; Stankus, 2009), the effect of the above described human impacts on twaite shad remains unsolved. Being an anadromous fish, the species is particularly vulnerable during the estuarine phase due to increased predation risk, diseases or the energetic cost of migrating and osmoregulatory abilities (Lochet et al., 2009). Hence, successful conservation and restoration of twaite shad populations requires insight into the effect of environmental conditions on spawning migration behaviour to aid successful reproduction.

Acoustic telemetry is a relative recent, but commonly applied technique to study fish behavior (Hussey et al., 2015). Fish are provided with an acoustic transmitter, which emits a signal with a unique ID code that can be detected by an ALS. This technique not only reveals the migration routes, but may also provide knowledge on the variables that influence migration and potential migration barriers when detection data, biotic and abiotic data are linked (Verhelst et al., 2018c). Surgical implantation is often used in tagging studies requiring pre- and post-operative care, anesthetics and confinement (Bridger and Booth, 2003; Huisman et al., 2016; Jepsen et al., 2005; Pauwels et al., 2014). Implantation has the potential to have both lethal and sublethal impacts on fish if performed incorrectly (Jepsen et al., 2002; Thiem et al., 2011). Due to the high sensitivity of twaite shad to handling and stress, surgical implantation could result in a high mortality and is therefore inadvisable. Rooney and

King (2014), for instance, stated that twaite shad exhibits an adverse reaction to handling and sedation and is therefore an unsuitable species for surgical implantation. Telemetry studies on allis (*A. alosa* Linnaeus 1758) and American (*A. sapidissima* Wilson 1811) shad have been conducted by means of gastric implantation of tags (Acolas et al., 2004; Dutterer et al., 2016; Frank et al., 2009; Olney et al., 2006; Tétard et al., 2016). Gastric implantation is a less invasive method than surgical implantation but it might result in regurgitation or mortality due to stomach rupture (Murphy and Willis, 1996; Nielsen, 1992). However, since twaite shad is more sensitive than allis and American shad (Baglinière and Elie, 2000), few telemetry studies have been conducted on twaite shad. Recently, Rooney et al. (2013) successfully applied external tagging on twaite shad in Ireland. Here, we present a protocol for external tagging of twaite shad, which is partly based on the method of (Rooney and King, 2014).

10.1.3 Material and methods

Study area

The River Schelde is 435 km long, originating on the plateau of Saint-Quentin in France. The Schelde estuary is approximately 160 km long and discharges into the North Sea. The estuary has a complete salinity gradient from polyhaline to a tidal freshwater zone, including extensive freshwater, brackish and salt marshes to its ecosystem (Fig. 10.1). It is a well-mixed estuary characterized by strong currents, high turbidity and a large tidal amplitude up to 6 m

(Seys et al., 1999). It can be divided in two sections (downstream to upstream): the Westerschelde (WS) in the Netherlands from Vlissingen to Zandvliet and the Zeeschelde (ZS) in Belgium, from Zandvliet to Gent. Further upstream the river is obstructed by sluices and weirs, which reduces tidal action and salt-water intrusion. Historical observations on the spawning sites of twaite shad in the River Schelde indicate they are located downstream of the first weir in the freshwater tidal reach of the watershed (Vrielynck et al., 2003). Therefore, in this study no physical migration barrier was encountered by the fish.

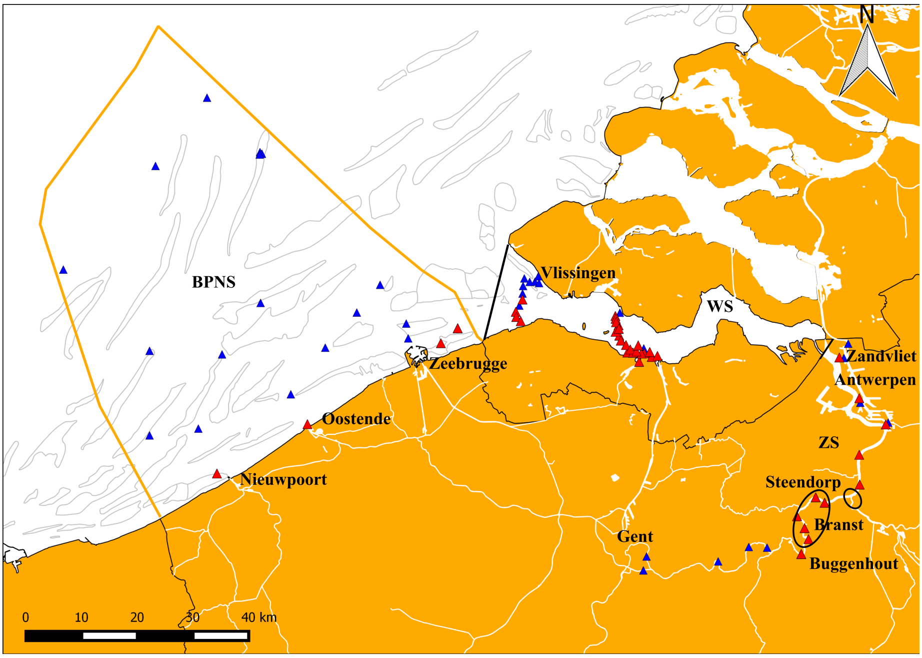

Figure 10.1: The locations of the acoustic listening stations (red triangles where shad were detected and blue triangles where shad were not detected) in the Belgian part of the North Sea (BPNS), Westerschelde (WS) and Zeeschelde (ZS). The borders between each of the three systems are indicated by a solid line. Spawning activity visually observed by people within a voluntary network, are indicated by circles.

ALS network

Within the framework of the LifeWatch observatory, a permanent acoustic network of 74 ALSs (VR2W, VEMCO Ltd, Canada) has been present since the spring of 2014 in the Zeeschelde (20 ALSs), Westerschelde (34 ALSs) and the

Belgian part of the North Sea (20 ALSs) (Section 2.3). They are moored at strategic locations to maximise the probability of detection. Hence, ALSs are deployed longitudinally in the Zeeschelde, in arrays in the Westerschelde and scattered in the BPNS (Fig. 10.1), thus covering around 180 km of river. The Belgian part of the North Sea stretches up to 81 km north and has a coastline of approximately 72 km long, covering a surface of 3454 km^2.

Tagging

Fish were caught in the Zeeschelde, near Antwerpen and Branst, with two midwater beam trawls from an anchored boat in April 2015 (Breine et al., 2015). Each trawl consisted of a net fixed between two eight meter long steel beams. The lower beam was dropped to the bottom of the river while the upper beam was held at the surface. Both ends of the beams were attached to the anchor that keeps the boat at a fixed place. Two nets were submerged for one or two hours during flood and ebb tide, respectively. Fish caught in the nets remain unharmed as flood tide prevents the nets from collapsing. Landed shad were checked for external damage and general condition (i.e. the capture effect). For each fish, total length (to the nearest mm) and weight (to the nearest gram) were measured, whereafter they were transferred to a 50 L oxygenated tank. Eight twaite shads were tagged with coded acoustic transmitters (V7 and V9, VEMCO Ltd, Canada), which emit signals at 69 kHz (Table 10.1). The tags had the capacity to emit signals for 2 (V7) and 4 (V9) months. The weight of the tags never exceeded 2% of the body mass of the captured shads (Jepsen et al., 2005).

To tag the captured fish, they were transferred to a surgery basin filled with

Table 10.1: Used tag types and properties.

Transmitter type	Number of shads tagged	Length (mm)	Diameter (mm)	Weight in air (g)	Weight in water (g)	Battery life (days)
V7	4	18	7	1.4	0.7	64
V9	4	24	9	3.6	2.2	132

sufficient aerated water to cover the head whilst exposing the dorsal surface. Fish were held partly under water with the head covered by a wet towel during the tagging procedure. Two to three scales were removed under the dorsal fin with a medical forceps to allow easy perforation of the two hollow needles (20G). A surgical thread (Ethilon) attached to the tag by a heat-shrinkable sleeve was passed through each needle so that they pass through the body of the fish while withdrawing the needles. A two mm thick rubber plate attached to the needles was then slid over the threads to reduce friction from the tag. Finally, the tag was drawn tightly against the dorsal fin and the plate was stabilized with two aluminum sleeves (Fig. 10.2). After tagging, the fish were placed back in the oxygenated tank for approximately 30 seconds until they started swimming. Then they were released into the Zeeschelde at their catch location. An overview of the tagging protocol is given in Table 10.2. Handling never took more than 90 seconds and could be reduced to this minimum by not anesthetizing the fish. The experiment was approved by the Ethical Committee of the Research Institute for Nature and Forest in Brussels (LA 1400559) and complies with the national legislation in Belgium transposing EU Directive 2010/63/EU (2010) on the protection of animals used for scientific purposes.

Figure 10.2: Dorsal view of fixed tag under the dorsal fin.

10.1.4 Results and discussion

In this study, we present a method for external tagging of twaite shad, adapted from a protocol described by Rooney and King (2014). The main difference with the method of Rooney and King (2014) was the attachment of the tag to the body to reduce friction. In Rooney and King (2014), the tag could freely move, which may enhance the chance of irritation. However, our results were similar to Rooney and King (2014) and in both studies, no recapture of tagged individuals occurred that would allow to draw conclusions about potential skin irritation due to tagging. Nonetheless, based on the telemetry results, twaite shads may not be strongly affected by the tags during the tracking period. In

Table 10.2: Procedure external tagging of twaite shad.

Preparation	1. Necessary material • Tag • Surgical thread (Ethilon) • Two hollow needles (20G) • Heat-shrinkable sleeve and aluminium sleeves • Rubber plate (2 mm) • Aerated tanks for surgery and recovery • Medical forceps 2. Prepare the tags by attaching a 20 cm long Ethylon thread to the tag with the heat-shrinkable sleeve. Each thread-end should later be passed through one of the two hollow needles that perforate the shad's dorsal fin.
Transfer caught fish	• Place shad in aerated tank and check its condition. • Evaluate the tag/fish weight ratio. • Transfer the shad to the surgery basin.
Position caught fish	• Position the fish with the dorsal fin upwards and above the water • Gently cover the head with a wet towel to reduce handling stress
Performing the surgery	• Remove two to three scales below the dorsal fin with a medical forceps. • Perforate the rubber plate with the two hollow needles. • Perforate the shad with two hollow needles just below its dorsal fin where the scales were removed. • Pass the surgical Ethilon thread through the hollow needles (one thread per needle) and pull the tag against the shad's body. • Slide the rubber plate over the thread to the shad's body. • Stabilize the rubber plate with two aluminum sleeves.
Transfer tagged fish	• Transfer the tagged shad back to the aerated tank to evaluate its welfare for approximately 30 seconds. • Release the tagged shad at the catch location.

total, 22 adult twaite shads (mean total length 39.7 cm, range: 33.9-47.3 cm) were caught during upstream spawning migration in the Zeeschelde near Antwerpen and Branst (Fig. 10.1). Two of these shads were injured after landing. Probably they got wounded by debris carried by the currents into the nets.

Eight of all caught twaite shads were acoustically tagged. One of these eight shads was never detected at a listening station, while two were detected at only one location for less than a day and 31 days, respectively. A plausible explanation would be that the tag was detached or the fish died, either as a result of the tagging procedure or by predation . Predation by cormorants (*Phalacrocorax carbo* Brisson, 1760), for instance, can certainly not be ruled out.

The remaining five tagged shads were detected at on average 21 ALSs (range 8-30 ALSs), and all together they were detected at 39 out of 74 ALSs between April 22th 2015 and June 28th 2015 (Table 10.3). Note that the tracking period varied among shad and lasted between 23 and 65 days. Specifically, the most upstream detection location was about 110 km upstream of the mouth of the estuary and the furthest detection location in the BPNS was near the coastline in Nieuwpoort, about 135 km from the catch location of the shad. Further, all five shad showed both upstream and downstream movement behaviour. The extent of their movements was similar to the study of Rooney et al. (2013), who found six of out eight shad that provided extensive tracking data. In the Zeeschelde, in 2015, twaite shad spawned in the tidal freshwater part between Buggenhout and Steendorp (90 km upstream) (visual observations).

We chose not to use anesthesia to reduce handling time, as this might be a crucial aspect to improve twaite shad survival. Notably, the effectiveness of a certain dose of anesthesia can vary according to the water temperature (Jepsen et al., 2002). Although Hao et al. (2006) and Ross et al. (1993) indicated a positive effect of anesthesia on American shad, we hypothesized that the recovery time from anesthesia of 6 to 7 minutes (Ross et al., 1993) would prolong the procedure too much, thus increasing the chance of death after release of the twaite shad (de Laak, 2009). The absence of anesthetics in the external tagging procedure of salmon (Thorstad et al., 2000) and the gastric implantation of tags in other shad species (e.g. Bailey et al. (2004) for American shad and Tétard et al. (2016) for Allis shad) might further support this. Nonetheless, the potential positive effect of anesthesia on twaite shad handling stress should be further investigated (Hao et al., 2006; Ross et al., 1993).

Table 10.3: Number of tagged twaite shad with tag type, total length (cm), weight (g), catch location and date, first and last detection, tracked time (days), number of locations and different areas where the fish were detected (Belgian part of the North Sea (BPNS), Westerschelde (WS) and Zeeschelde (ZS)).

Length (cm)	Weight (g)	Catch location	Catch date	First detection	Last detection	Tracked time (days)	Acoustic Listening Stations (ALSs)	Areas
35.2	378	Antwerp	23rd April	24th April	17th May	23	8	ZS - WS
37	456	Antwerp	23rd April	24th April	28th June	65	30	ZS - WS - BPNS
34.5	360	Branst	22nd April	22nd April	17th May	25	21	ZS - WS
45.6	822.4	Branst	22nd April	22nd April	17th May	25	22	ZS - WS
33.9	325	Branst	22nd April	22nd April	22nd May	30	26	ZS - WS - BPNS
35.3	365.6	Branst	22nd April	23rd April	17th May	0	1	ZS
40.5	591.1	Branst	22nd April	NA	NA	0	0	NA
44	800	Branst	30th April	30th May	31st May	31	1	ZS

10.1.5 Conclusion

The new external tagging technique is promising as it did not prevent tagged shads from extensive up- and downstream migrations in the Schelde estuary and the BPNS for a period up to two months. Since tagged shads were not recaptured, it is unknown to what extent external tags could affect the fish's physiology and movement behaviour. Therefore, further research can help to understand the direct effects on the shad's welfare, so that the method can be further improved and applied to other *Alosa* species as well. In this respect, further research on the effect and doses of anesthesia, and of handling and tagging twaite shads is strongly encouraged. Tagging more individuals, accompanied by laboratory monitoring of tagged fish, could reveal important information about movement behaviour, tag loss, lesions and infections. Telemetry studies on a larger number of twaite shad are important, because they can provide essential information on the shad's spatio-temporal behaviour, which may well be important for the establishment of successful species management and conservation plans.