# Evaluation of surgery procedures for tagging eel *Anguilla anguilla* (L.) with biotelemetry transmitters

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#### Abstract

Externally attached telemetry transmitters are unsuitable to tag yellow eels Anguilla (L.), in streams where they exhibit cryptic life habits and hide in narrow cavities between rocks. We evaluated the adequacy of surgical implantation and closing procedures for tagging eels with biotelemetry transmitters. Epoxy dummy transmitters  $(18 \times 8 \text{ mm}, 1.6-1.7 \text{ g})$  were implanted in eels anaesthetised with 2-phenoxy-ethanol  $(0.9 \text{ ml } 1^{-1})$ , through a 20 mm mid ventral incision made in the posterior quarter of their body cavity. The incision was either left open, or closed in different ways: stitches (absorbable or non absorbable suture material) or commercial-grade cyanoacrilate adhesive (Loctite<sup>TM</sup>). Fish were stocked in a  $4 \text{ m}^2$  flow through tank (15-17 °C), controlled daily for mortality and weekly for evaluating the healing process.

No transmitter was expelled over a 12-week period, even in eels with unclosed incisions, of which 50% healed within 28 days ( $t_{50}$ ). Regardless of the nature of the filament, suturing induced skin and muscle necrosis, caused significantly higher mortality rates (60% after 10 weeks) and paradoxically slowed down the healing rate (40 and 45 d, respectively). Cyanoacrilate suppressed the inflammatory response and granted higher survival rate (90%), but did not permit to speed up the closing process ( $t_{50} = 52$  d), as eels actively bit and removed the adhesive within hours. This behaviour was suppressed when we applied a freshly cut fragment of the eel dorsal fin as a biological bandage over the drying cyanoacrilate. The adhesive remained in place for one to two days and permitted to substantially increase the healing rate ( $t_{50} = 15$  d). These results substantiate the efficiency of surgery techniques for tagging eels with radio transmitters, at least for units of small weight and bulk.

## Introduction

Among the range of techniques available for marking or tagging fish, telemetry tags undoubtedly represent the most powerful tools as they permit to track the movements of the fish, and provide information on its environment or physiology, when appropriate sensors are coupled to the transmitter (Winter, 1983; Priede & Swift, 1992; Baras & Lagardère, 1995; Baras & Philippart, 1996). However, telemetry tags have a substantial size and weight (> 0.8 g and > 1.3 g in the air, for radio and acoustic tags, respectively), due to the electronic circuitry and battery required to power the acoustic or radio signal. These features limit the weight range of fish to be tagged with telemetry tags,

and imply a fine tailoring of tagging procedures to avoid any major bias resulting from transmitter attachment or presence on, or inside the fish (syntheses in Summerfelt & Smith, 1990; Baras, 1991; Baras et al., 1998).

These considerations may account for the relatively small number of telemetry studies on eels (*Anguilla* spp., see review in Nielsen, 1988). Externally attached transmitters have been frequently used to tag large eels during their movements in lakes, estuaries, tidal creeks and the first stages of their marine migration on the continental shelf (Tesch, 1974, 1989; Helfman et al., 1983; McGovern & McCarthy, 1992). They are known to modify the hydrodynamism of the

fish (Tesch, 1974) and may be unsuitable to study resident life stages in streams, where eels exhibit cryptic life habits and hide in narrow cavities in between rocks. Intragastric transmitters are prone to regurgitation by eels (Stasko & Rommel, 1974) and, when retained, might interfere with the appetite of eels as they modify the degree of stomach fulness. Surgically implanted tags may prove the best technique for long term tagging of yellow eels, although this technique has rarely been used and evaluated. LaBar et al. (1987) tracked European eels Anguilla anguilla (L.) with internal tags in a Spanish lake but gave no information on the tagging procedure and effects in the long run. Ernande (1995) noted that acoustic transmitters, with weight in the air circa 3% of the body weight of A. anguilla, might caused a deformation of the body wall and were systematically expelled within two weeks. This expulsion process may be the consequence of inappropriate incision closing procedures in a species with a narrow body cavity and anguilliform swimming mode, which may both cause outwards pressure and cause the expulsion of the transmitter before the incision has healed.

As a preliminary step to a tracking study on the behavioural ecology of yellow eels *A. anguilla* in Belgian streams, we evaluated the feasibility of tagging eels with radio transmitters implanted into the intraperitoneal cavity, and the relative efficiency of different closing procedures.

#### Material and methods

The study was conducted on wild eels, captured in fish passes on the River Meuse. We used epoxy (Epon 812) constructed dummy transmitters that were similar in weight and bulk to commercially available motion-sensitive transmitters (selected model: ATS, Inc. 377,  $16 \times 8$  mm in diameter, 1.6-1.7 g in air). This model weighed less than 0.6% of the body weight of the eels in this experiment (285–958 g), and its short length (comparatively to the body length of eels: 562-780 mm) was supposed to impose no major restriction on their swimming behaviour.

Eels were anaesthetised with 2-phenoxy-ethanol (0.9 ml  $1^{-1}$ ), reached anaesthesia stage III.2 (Mac-Farland & Klontz, 1969) after 6 to 8 minutes then were placed ventral side up into a half cylindrical support, using wet paper to adjust the position of their body into the support. A 18–20 mm long incision was made with a scalpel on the mid-ventral line, in the

posterior quarter of the body cavity. The dummy transmitter was inserted cranially and positioned 3 to 4 cm forth of the incision with a plastic plunger, in order to minimise the risk of inside out pressure during the healing process. Two closing procedures were evaluated against a control procedure, where the incision left open, as it is frequently for small implants such as passive integrated transponder (PIT) tags (Prentice et al., 1990).

In two groups of eels (2a and 2b), the incision was closed up with two stitches 6–7 mm apart, using 2 Dec filaments (absorbable plain catgut or non absorbable polyamide monofilament) that were fixed to 16 mm cutting needles. Cutting needles were preferred to atraumatic needles accordingly with the conclusions of Thoreau and Baras (1996) for species with strong body walls, like eels. In group 3a, the incision was closed with a commercial grade cyanoacrilate adhesive (Loctite<sup>TM</sup>). Accordingly with the recommendations of Nemetz and MacMillan (1988), the two edges of the incision were blotted dry and maintained together by exerting a lateral pressure over the body wall of the eel during the application and drying of the adhesive (*ca.* 1 min).

All tagged eels were stocked together with a group of 15 control fish in the same flow-through tank  $(4.0 \times 1.0 \times 0.5 \text{ m}^3)$ , in the Fish Research Station of the University of Liège, in Tihange. In order to mimic the spring thermal regime of Belgian waters, the water temperature in the tank was maintained at  $16 \pm 1$  °C throughout the experiment, by mixing waters from the River Meuse and from the effluent of the Tihange nuclear power plants. Dry food pellets were distributed by an automatic feeder. The tank was checked daily for fish survival. Eels were checked weekly for transmitter retention and for evaluating the healing process. It was initially programmed to analyse the growth of eels depending on the tagging procedure, but it rapidly turned out that all eels, including those of the control group, ate very little in captivity at  $16 \pm 1$  °C and were gaining no weight. Transmitter retention was checked by a gentle pressure over the abdomen of the fish and empirically by search for lost transmitters on the bottom of the emptied tank. The healing stage was evaluated by testing, with a metal probe, which layers of the body wall had begun tissue reconstruction. The incision was considered as healed when the dermis and epidermis had started closing up over the whole length of the incision.

Transmitter retention rates and fish survival rates were compared by contingency table analyses. Healing rates were compared by Mann-Whitney *U*-tests. Null hypotheses were rejected at the 0.05 level of significance.

## **Results**

No transmitter was expelled over a 12 week period, even in the group where the incision was not closed. Regardless of the nature of the filament, suturing caused deep cuts into the body wall of most eels. From the second week onwards, most cuts degenerated into necrotic tissue, and infections were observed at these sites. The absorption of catgut filaments during the third week, or the removal of the permanent polyamide filaments after two weeks, limited the extents of the cuts and necrotic tissue, but about 50% of the sutured eels died within the five first weeks. Surviving eels healed their incision within 6–7 weeks (Table 1).

Eels with incision left open healed much faster and their survival rate was similar as in the control group. Inflammatory responses were observed on the edges of the incision but vanished within the first three weeks. The use of cyanoacrilate apparently suppressed the inflammatory response at the incision site and slightly further improved the survival rate (90% after ten weeks). However, it did not speed up the closing process, as eels required an average time of 52 days to heal their incision. Additional observations in aquarium on the behaviour of tagged eels closed up with cyanoacrilate revealed that the adhesive was shed within hours, not as a consequence of tensions caused by swimming, but because eels were actively removing it by biting. In order to possibly modify this behaviour, we refined the cyanoacrilate closing procedure by applying a freshly cut fragment of the eel's dorsal fin over the drying cyanoacrilate (group 3b, Table 1). Presumably due to the presence of this biological bandage over the incision, the eels did not actively remove the adhesive, which remained in place one or two days, then was shed. This procedure permitted to reduce the healing time to 15 days. Only one eel from group 3b died, after its incision had healed. The dissection of tagged eels three to four months after tagging indicated that most (90%) implants had moved posterior to the incision but had not caused internal damage.

## **Discussion**

The results indicated that surgical implantation was an adequate procedure for tagging eels with biotelemetry tags, at least when adequate closing procedures were used. Eels with unclosed incisions had a high survival, maximum retention rate and healed their incision within a month. These observations may somehow question the relevance or necessity of closing procedures, especially when dealing with short incisions comparatively to the body length of the animal. Additional experiments with PIT tags surgically implanted into the body cavity of eels also provided evidence that the non closing procedure was adequate, as 5-6 mm incisions completely healed within 17 days at 16±1 °C. Although this procedure would undoubtedly be initially challenged by those responsible for enforcing animal welfare legislation, this study indicated that it granted far better survival rates than with suturing, which is the most popular and recommended technique for closing abdominal incisions in fish (e.g. Hart & Summerfelt, 1975; Baras et al., 1998). This finding is consistent with studies on liver biopsy in channel catfish Ictalurus punctatus Rafinesque (Carmichael, 1991), on abdominal implants of radio tags in the European barbel Barbus barbus (L.), (Baras, 1992) and of PIT tags in juvenile fishes (salmonids: Prentice et al., 1990; tilapia Oreochromis niloticus (L.): Baras et al., 1996), which proposed that closing incisions with stitches did not substantially speed up the healing process. In this study, it appeared that suturing induced skin and muscle necrosis, caused significantly higher mortality rates and slowed down the healing process comparatively to unclosed fish, presumably as a result of general low condition. This effect of stitches on the recovery of eels is a further example to the potential detrimental effect of transcutaneous foreign bodies that have been reported by several authors (Roberts et al., 1973; Marty & Summerfelt, 1990; Baras, 1992; Knights & Lasee, 1996), and which presumably reaches its climax in fish species of which the skin is not covered by scales.

In comparison to the above procedures, the use of cyanoacrilate in eels suppressed the inflammatory response at the incision site, as observed in other fish species (Nemetz & MacMillan, 1988; Petering & Johnson, 1991). However, the healing rate of eels closed with cyanoacrilate was about twice as long as in unclosed eels, presumably because the removal of the adhesive by biting, within the hours following the surgery, caused additional lesions in the incision

Table 1. Survival and healing of surgically implanted eels, depending on the closing procedure. $t_{50}$
is the time of complete healing of 50% of the surviving fish. Values sharing at least one common
upperscript label do not differ at the 0.05 level of significance (contingency table analyses for survival
rates; Mann-Whitney $U$ tests for healing)

			Survival (%) after n days				Healing (days)
Group	N	Closing procedure	7	28	49	70	<i>t</i> <sub>50</sub> (range)
Control	15	_	100 <sup>a</sup>	100 <sup>a</sup>	93 <sup>a</sup>	87 <sup>a</sup>	_
1	10	none	100 <sup>a</sup>	$80^{a,b}$	80 <sup>a, b</sup>	80 <sup>a,b</sup>	28 (18–35) <sup>a</sup>
2a	10	suture, catgut	100 <sup>a</sup>	60 <sup>b</sup>	50 <sup>b</sup>	40 <sup>b</sup>	40 (24–46) <sup>a,b</sup>
2b	15	suture, polyamide	73 <sup>b</sup>	53 <sup>b</sup>	47 <sup>b</sup>	40 <sup>b</sup>	45 (36–48) <sup>b</sup>
3a	10	cyanoacrilate	100 <sup>a</sup>	90 <sup>a</sup>	90 <sup>a</sup>	90 <sup>a</sup>	52 (16–70) <sup>a,b</sup>
3b	10	cyanoacrilate + bandage	100 <sup>a</sup>	100 <sup>a</sup>	90 <sup>a</sup>	90 <sup>a</sup>	15 (14–28) <sup>c</sup>

zone. The addition of a biological bandage caused the adhesive to remain in place for a few days, which was a sufficient delay to permit the adjustment of the edges of the incision and to speed up the first steps of the closing process. This 'bandage' technique could be worth testing in other fish species, especially in species without scales, where suturing could prove detrimental.

No transmitter expulsion was observed in this study, even in eels of which the incision had not been closed at all. In a recent field study (Baras et al., 1998), all radio-tagged eels released in a small stream with rock substrate survived and retained their 1.6 g transmitter until the end of the battery life. This study further documented that tagged eels could clear small weirs within their home range, suggesting that the presence of the tag did not cause any major impairment of their mobility or swimming capacities. These observations suggest that incision exits observed in past studies (Ernande, 1995) originated from the use of tags with excessive weight or bulk (transmitter to body weight ratios in the air from 2.4 to 3.0% in Ernande's study). A ratio of less than 0.6% obviously limits the risks of expulsion and permits to study the behaviour of eels in all types of ecosystems and environments. However, it reduces the weight range of eels which could be tagged with intraperitoneal transmitters (over 130 or 215 g, for radio and acoustic tags, respectively). The small battery size further reduces the reception range and/or tag endurance, and and imposes additional restrictions for studies using internally tagged eels. Endurance could be substantially improved by using transmitters operating on duty cycles, i.e. from dusk to dawn as eels essentially are nocturnal fish (LaBar et al., 1987; McGovern & McCarthy, 1992;

Baras et al., 1998). A possible alternative to programmable transmitters would consist in adapting the shape of the transmitter to the narrow body cavity and swimming of eels, e.g. by designing a flexible long transmitter with narrow diameter, of which the batteries and electronic circuitry would be linked by flexible connectors.

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