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Behavioural effects of surgically implanting transponders in European eel, *Anguilla anguilla*

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Key words: behaviour, telemetry, surgical implantation, methodological effects, eel.

Abstract

The applicability of using transponders (Nedap Trail™) to tag European eel *Anguilla anguilla* (L. 1758) during their silver eel stage, was tested in a controlled tank experiment. Most studies on effects of implanted radio tags focus on the impact on mortality, disease, wound healing and growth. In this experiment we also measured individual activity continuously, which allowed to detect more subtle effects on behaviour as well. In total 40 silver eels (680-1685 g) were anaesthetized and injected with a micro PIT-tag. In 20 of these, dummy transponders similar in shape (cylindrical, 14x63 mm), weight (26.5 g in air, 16.0 g in water), volume (9.5 cm^3) and surface (glass) to Nedap-transponders were surgically implanted in the body cavity. All eels were placed in a Migromat®-tank with five compartments connected with openings that allowed free movement between compartments. Each opening was covered by an antenna recording individual passages. Because the tank was placed in the open, had transparent lids and was flowed with river water, eels could respond to 'natural' environmental stimuli, e.g. water temperature, turbidity, light and moon phase. The experiment was ended after 11 weeks (October-December). There was no significant difference in mortality rate between the control (15%) and experimental group (10%). All eel in the experimental group had closed wounds and none of the transponders was shed, nor any sign of expulsion was observed. In both groups one PIT-tag was lost. Individual activity level of the remaining eels was 38% lower in the experimental (n=17) than in the control group (n=16), indicating at least some effect of implanting transponders on the behaviour of eel. There was, however, no difference between groups in the timing of activity, neither during the entire period nor diurnal. Consequences for the interpretation of field telemetry experiments are discussed.

Introduction

Biotelemetry is a fast developing technology that is increasingly used to study fish behaviour (Lucas and Baras, 2001). In field experiments measurements are taken from tagged fish only, without the possibility of a control group. Therefore, it is important to determine potential bias in behaviour induced by the tagging procedure and materials on forehand in controlled experiments, especially since large differences in response to applied methodology are indicated for different species, life stages, and environmental circumstances (Jepsen *et al.*, 2002). Most controlled experiments on the effect of applying telemetric tags, however, focus on effects such as mortality, disease, wound healing, and growth (e.g. Lucas, 1989; Martin *et al.*, 1995; Martinelli *et al.*, 1998; Walsh *et al.*, 2000), whereas experiments with behavioural measurements are relatively few (Moore *et al.*, 1990; Adams *et al.*, 1998; Perry *et al.*, 2001; Koed and Thorstad, 2001).

In the present study we focus on the possible effects of surgically implanted transponders on the behaviour of the European eel *Anguilla anguilla* (L. 1758) by means of a controlled tank experiment. In addition to more traditional parameters we also continuously recorded individual movements to detect possible differences in the timing and level of activity.

This study was performed within the context of a project to determine activity time patterns and serial mortality rates due to hydropower and fisheries in the River Meuse, the Netherlands (Bruijs *et al.*, 2003).

Materials and methods

Test fish and handling procedures

Eels were caught with fykenets by a professional fisherman at 8-9 October 2001 in the river Meuse at Ohé en Laak, The Netherlands. They were kept in oxygenated basins between catch and treatment on 10 October 2001. Only eels with a completely silver white ventral side were used, rejecting individuals with yellow or partly yellow ventral sides. We used 40 eels between 52.5 and 91.0 cm total length and 680 and 1685 g. Males do not grow that large before migrating (Dekker, 2000), thus all used fish were females.

Surgical implantation technique

All individuals were anaesthetized with 2-phenoxy-ethanol (0.9 mll^{-1}), weighed (g), measured (mm total length) and subcutaneously injected with an individual coded PIT-tag (Passive Integrated Transponder, TROVAN®) in the dorsal muscle near the head. In 20 eels, dummy transponders similar in shape (cylindrical, 14x63 mm), weight (26.5 g in air, 16.0 g in water), volume (9.5 cm^3) and surface (glass) to Nedap Trail™ transponders (Breukelaar *et al.*, 1998) were surgically implanted in the body cavity by making a mid ventral 2-3 cm incision in the posterior quarter of the body cavity. The used surgical procedure was the best among five different procedures tested for European eel by Baras and Jeandrain (1998). The incision was closed by commercial-grade cyanoacrilate adhesive (Loctite™) and a freshly cut 3-5 mm wide fragment of the eel's dorsal fin was applied over the drying adhesive to act as a biological bandage over the incision. Surgery lasted 3-5 minutes at a water temperature of 15 °C. Eels were observed in a recovery tank until swimming behaviour reappeared and then released in the test tank.

Tank experiment

The experimental group with implanted transponders (n=20, length range 67-91 cm, mean±s.d.: 77.6±5.9 cm) and the control group (n=20, length range 53-90 cm, mean±s.d.: 66.3±8.8 cm) were placed together in one tank in order to avoid a possible tank effect. This Migromat® tank contained 5 m^3 water and consisted of five compartments connected by four square openings of 30 cm. Each of these openings was covered by an antenna loop that continuously registrated each individual passage (Adams and Schwevers, 1999; Fig. 1). Eels were free to move between the five different compartments and were found to do so, e.g. most eels visited several compartments more or less evenly distributed over the entire tank. The experiment was carried out from 10 October to 16 December, during the period of downstream migration, in the open field, with transparent lids and flowed with river water from the River Meuse, which enabled a response to 'natural' environmental stimuli, e.g. water temperature, turbidity, light and moon phase. A sediment layer of ca. 20 cm built up in the course of the experiment. During inspections dead or dying eels were removed from the tank. After 11 weeks at the end of the experiment, all remaining eels were checked and the presence of PIT-tags was determined. Eels of the treatment group were inspected for external and internal wound healing and the presence and location of the transponder. By external inspection it was determined whether the abdominal tissue or skin had closed, i.e. only scar tissue was visible. By internal inspection, after dissection, it was examined whether the transponder was still present, whether signs of expulsion were present, i.e. transponder enclosed in tissue, and what organ tissues were eventually grown to the abdominal tissue of the healing wound. Of all eels with implanted transponders pictures of the external and internal wound healing state were taken, allowing for later analysis of additional parameters.

Data analysis

Differences in mortality percentage between the experimental and control group were analysed using a *G*-test of independence (Sokal and Rohlf, 1995).

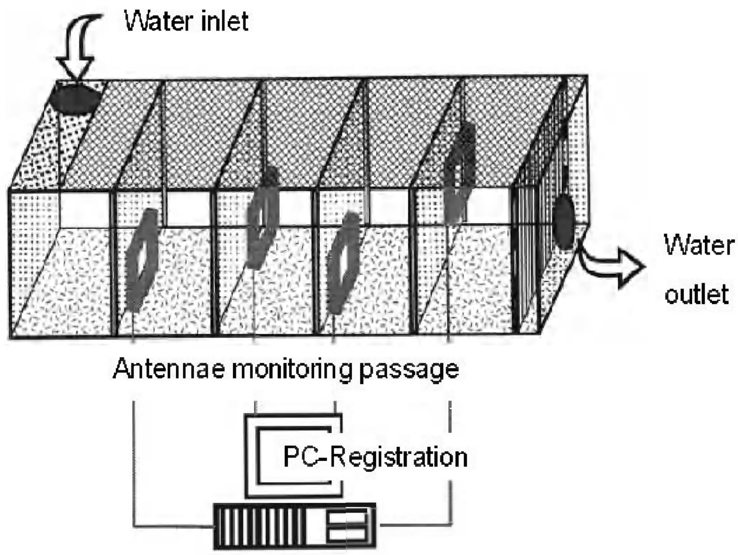

Fig. 1 – Schematic overview of the Migromat tank (1 m^3 water per compartment, 5 m^3 in total). The compartments are connected with openings covered by antenna loops that continuously monitor each passage of eels that move from one compartment to another.

For each individual, the number of passages through antenna loops was used as a measure for activity.

Because, unintentionally, the length distribution of the control group differed from the experimental group, we had to account for a possible length effect on activity when examining the effect of surgically implanting transponders. For this, we used a generalized linear model (GLM) that explained variance in activity for group, length and interaction between these two variables in the experimental tank:

$$A_{ij} = \mu + G_i + L + G_i{*}L + \varepsilon_{ij} \qquad (1)$$

where: A_{ij}=activity level of an individual eel (j), μ=mean activity, G_i=group (i=control, experimental), L=total length (cm), and ε_{ij}=error term.

However, due to the high colinearity between group and length, which are therefore not independent, in addition we used alternative tests for different groups of eels to examine a possible length effect on activity level. First, we examined the effect of length on activity by using data from an adjacent identical Migromat® tank, which will be further referred to as tank B where the experimental tank above will be referred to as tank A. Tank B was used for another experiment during exactly the same period (10 October-16 December 2001) to compare timing of activity in tanks with field data beyond the scope of this paper. However, the eels (n=30, length range 41-84 cm, mean±s.d. 66.4±11.5 cm) stocked in tank B were caught, handled, treated and implanted with a PIT-tag identically as described above for the control group in tank A. The following GLM model was used to examine the effect of length on activity in tank B:

$$A_j = \mu + L + \varepsilon_j \qquad (2)$$

where: A_j=activity level of an individual eel (j), μ=mean activity, L=total length (cm), and ε_j=error term.

Second, model (2) was applied to examine a possible length effect within the control group and experimental group of tank A, separately. Third, to compare a possible length effect between tanks, we examined a tank effect in combination with a length effect on the activity of eels that were given the same treatment, i.e. the control group of tank A and all eels in tank B, by using a GLM that explained variance in activity for tank, length and interaction:

$$A_{kj} = \mu + T_k + L + T_k{*}L + \varepsilon_{kj} \qquad (3)$$

where: A_{kj}=activity level of an individual eel with PIT-tags and without surgical implanted transponders (j), μ=mean activity, T_k=tank (k=tank A, tank B), L=total length (cm), and ε_{kj}=error term.

For the eels with implanted transponders in tank A we examined possible effects of external and internal wound healing state on activity by classifying them on the occurrence or absence of inflammation E_l (as a measure for external wound healing state) and the occurrence or absence of organ tissue grown to the healing wound I_m (as a measure for internal wound healing state). For this, variance in activity was tested with a GLM similar to model 2, where the parameter L was subsequently replaced by E_l=external healing (l=presence, absence of inflammation) and I_m=internal healing (m=presence, absence of tissue grown to wound tissue). For all GLM models, residuals were checked by a Shapiro-Wilk and Kolmogorov-Smirnov test on normality and interaction terms were removed from the models when these were not significant.

The timing of activity between the control and experimental group in tank A was examined on two different time scales. To compare timing between groups throughout the experiment, for each day the total number of antenna passages per group was determined. To compare timing between groups on a diurnal basis, for each of the 24 hours during the day the average number of antenna passages per group was determined. The correlation between groups was then determined for each of these two time scales (Sokal and Rohlf, 1995).

All statistical analyses were performed with SAS (version 8) software using α of 0.05.

Results

Mortality, disease, wound healing and tag retention

During the experiment from each group in tank A one eel died. In addition, one eel from the experimental group and two eels from the control group were removed, because they were near dead as a result of *Saprolegnia* sp. infection. Thus, total mortality rates were 10% and 15% for respectively the experimental and the control group, though not significantly different. In both groups at the end of the experiment, one healthy eel was found to have lost its PIT-tag, presumably during one of the first days after release in the tank since detections ceased shortly thereafter.

Within the treatment group at the end of the experiment, for all remaining 17 eels the wounds had closed, varying from completely healed with only scar-tissue visible to a closed abdomen but unhealed skin (Fig. 2). In 9 eels some organ tissue, mainly fat or intestine tissue was grown to the healed abdominal tissue. Inflammations were observed in 7 individuals. None of the eels had lost their transponder, which were all free laying in the body cavity and no signs of expulsion were observed.

Effects on activity level

Activity per eel in tank A was higher in the control group than in the experimental group (Fig. 3). When accounting for a group and length effect on activity level no significant effect was found for each of these parameters (model 1: group p=0.09, length p=0.91). Because group and length were not independent, we further examined a possible length effect on activity level. In tank B, where the length range was larger than in tank A, no significant effect of length on activity level was found (model 2: p=0.95). Also when testing the effect of length within the experimental group and the control group separately, no significant length effect was found (control: p=0.90, experimental: p=0.94). In addition, when combining all eels with only PIT-tags

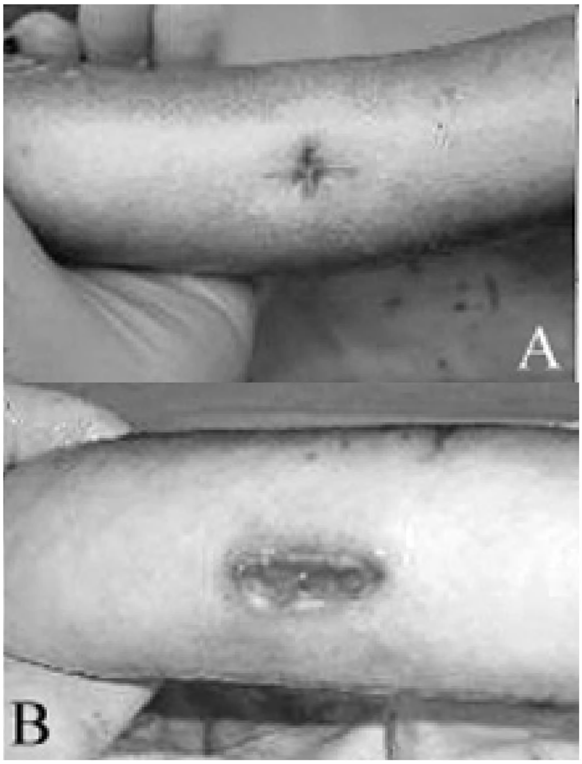

Fig. 2 – The range of wound healing state after 11 weeks. A: best healing, only scar tissue visible, B: worst healing, closed abdomen tissue but inflamed wound.

of tank A and B (model 3), again no significant effect of length was found (p=0.89). However, there was a significant effect between tank A and B (d.f.=2, F=4.25, p=0.05), where the average activity level of tank B was 36% lower than the control group in tank A, perhaps related to the number of eels in each tank (n=40 in tank A, n=30 in tank B). Because neither in tank B, nor in each of the groups in tank A, nor when combining the PIT-tagged eels of both tanks any indications for a length effect on activity were found, it appears justified to remove the length term from model 1. Then a significant effect of implanting transponders on the activity level was found (d.f.=1, F=5.44, p=0.03) where the model explained 15% of the variance. The average level of activity of the experimental group was 38% lower than of the control group.

When comparing average individual activity per group throughout the duration of the experiment (Fig. 3), several periods with different activity levels can be distinguished. The first 24 hours of the experiment, activity was nearly the same for each group. During 11 October-11 November, activity was relatively high in both groups with a peak on 12 October, and 38% less in the experi-

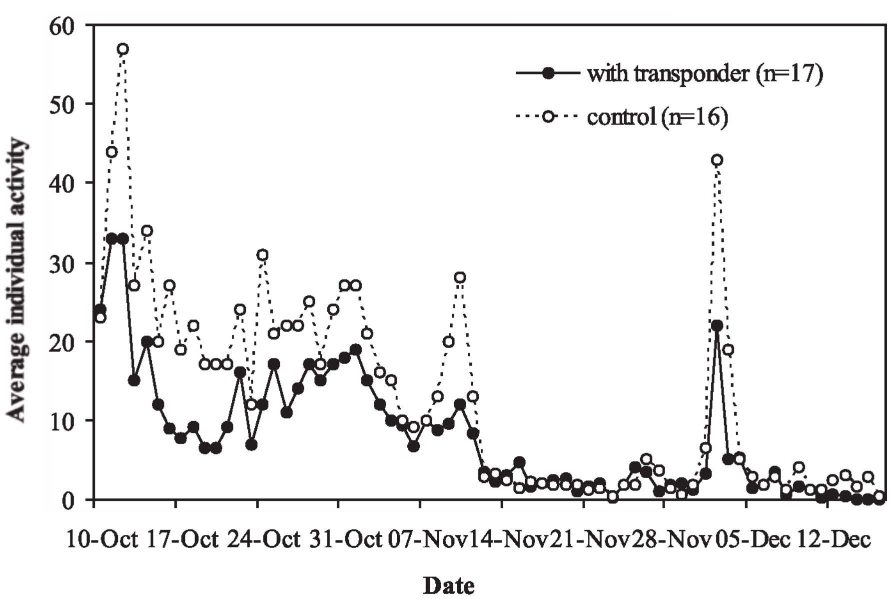

Fig. 3 – Average individual activity level per day for the experimental group with surgically implanted transponders and the control group during the entire period of the experiment in tank A from 10 October – 16 December 2001.

mental group. During 12-30 November, activity was very low for both groups and 10% higher in the experimental group. During 1-4 December, activity was high with a peak on 2 December, and 52% less in the experimental group. From 6-16 December activity was very low, and 55% less in the experimental group.
Within the experimental group, we did not find significant differences in activity patterns between eel with and without organ tissue growing onto the abdominal tissue (internal wound healing state, p=0.57) nor between eel with or without inflammations (external wound healing state, p=0.78).

Effects on timing of activity
When comparing average activity level of both groups during the day, a clear diurnal pattern was observed (Fig. 4). At night, especially between 18:00 and 22:00 hours, an increase in activity was observed, peaking at 20:00 hours. In the first five hours after sunset almost 40% of all activity took place. Very similar patterns were observed in all periods, also in the period when eel were relatively inactive (Fig. 3). Correlation between the activity level of the control and experimental group was highly significant both per day throughout the entire period (R^2=0.93, p<0.001) and diurnally per hour (R^2=0.95, p<0.001, Fig. 5).

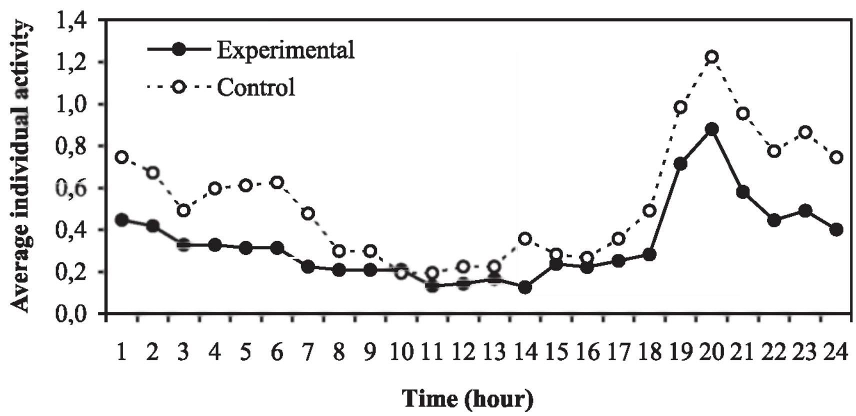

Fig. 4 – Diurnal patterns expressed as average individual activity level per hour during the entire period for the control group (broken line) and the experimental group (solid line) in tank A.

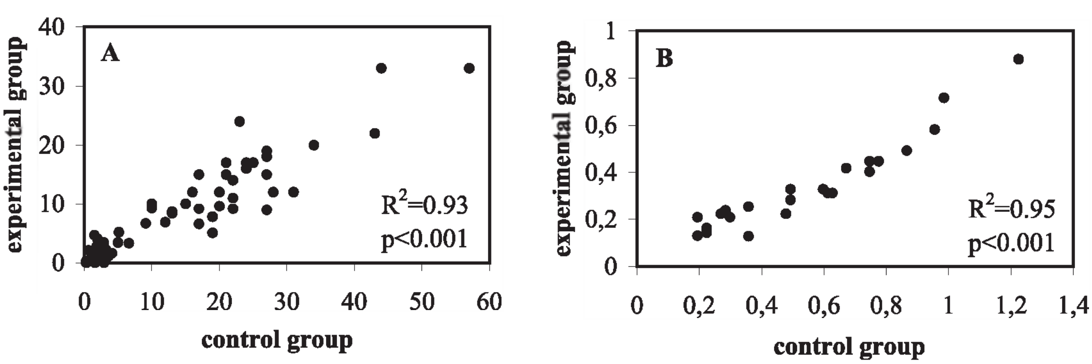

Fig. 5 – Correlation diagrams of mean activity level of the experimental group versus the control group in tank A for: A) each day during the entire period, B) each hour during the day (diurnal pattern).

Discussion

Because mortality rate was similar, even slightly less, in the experimental group than in the control group, and for the experimental group all wounds had closed abdominal tissues and no transponder loss or expulsion was observed during the 11 week period, it might be concluded by these commonly used criteria that surgically implanted Nedap-transponders are suitable to study silver eel migration.

When looking at the behaviour of eels in more detail, the timing of eel activity was very similar between both groups during the entire period as well as diurnal. Activity level, however, was 38% lower for the experimental than for the control group. Because no indications for a length effect on activity were found in tank B, within each group in tank A, or for all eels where no transponders were surgically implanted of tank A and B combined, it is unlikely that the uneven length distribution caused the differences between the experimental and control group in tank A and that the found effect was related to the surgical implantation of transponders. These eels were apparently less active. For this, we discuss two possible explanations.

First, the surgery and subsequent healing of the wound might have resulted in a lower activity level. If recovering from the treatment would be the main cause for the difference in activity levels between the two groups, it was expected that the impact decreased in time. The difference between both groups, however, showed no such trend, and the relative difference was even higher in the last part of the experiment (Fig. 3). Furthermore, no effect of wound healing state on activity level was found within the experimental group. It is therefore unlikely that this is the major cause for the observed difference.

Second, carrying the transponder may have inflicted lower activity. If this is true, it would be expected that the effect was relatively constant in time, which is close to what was observed. The transponder/body weight ratio was relatively high, ranging from 1.4 to 3.7%. In most eels it was higher than the often recommended 'Winter's 2% rule' (Winter, 1996). Thus, in the individuals with a high ratio, i.e. the smaller individuals, it would be expected that the activity was lower. However, within the experimental group no effect of length was found. It might be that the effect in eels already occurs at ratio's far lower than 2%. More studies have questioned this general rule of thumb (Jepsen *et al.*, 2002), where sometimes effects well below 2% were found, and in other studies no effect could be demonstrated on behaviour in salmon smolts with ratios up to 10.7% (Brown *et al.*, 1999). It is likely that eel, given its elongated body form and relatively narrow body cavity, flexible locomotion mode, efficiently fine tuned swimming endurance during the silver eel stage is relatively susceptible to carrying a transponder. We share the opinion of Gallepp and Magnuson (1972) and Jepsen *et al.* (2002) that it is unlikely that recommendations for a single weight ratio would be suitable in all species, or even within species. Some species and sizes of fish may be able to compensate for additional weight far better than others. But not only weight of the transponder might influence behaviour, volume might, especially for eel, be even more important. The relatively large transponder used in this experiment may fill much of the body cavity putting pressure on the internal organs, even though silver eel is supposed to cease feeding. Moreover, because of their anguilliform swimming mode, the relatively high transponder/body length (6.7-9.2%) might hamper their movements. Also, transponders proved to have detrimental effect on vertical stationing (Greensted and Morgan, 1989). Buoyancy is an important parameter, where the degree to which this can be compensated by means of filling the swim bladder varies between species (Lefrancois *et al.*, 2001).

Within the current experiment, it can only be indicated whether the surgery treatment or carrying the transponder are more plausible explanations underlying the found effect on activity level as described above. However, to disentangle the effects of the surgical treatment and carrying the transponder, additional experiments are required that also include a 'sham'-tagged group, undergoing the same surgical procedure but without actually implanting a transponder (Brown *et al.*, 1999). In contrast to effects on the level of activity, no effects on the timing of activity were found. Both groups showed very similar patterns throughout the experiment and throughout the day. The very high activity during the first days of the experiment might be attributed to adjusting to the tank. The prolonged occurrence of higher activity thereafter, until 12 November, is probably at least partly due to a reaction on external stimuli, because commercial catches of silver eel in the River Meuse were also high during these weeks (Bruijs *et al.* 2003). The observed peak in early December is probably also initiated by external stimuli, although no commercial catch data of this week was available. Eels proved to be essentially nocturnal (Muller, 1972) and activity is normally peaking in the first two hours following sunset (Hain, 1975). This diurnal pattern is clearly reflected by the results of this tank experiment. Both groups tend to show rather 'natural' behavioural patterns in relation to external stimuli.

Implications for field telemetric studies

Studying timing and onset of downstream migrating silver eel in relation to trigger factors or environmental cues with Nedap-transponders seems to be justified, because no effects on timing of activity or mortality were observed. Given the lower activity of eels implanted with transponders one should be aware that at least some bias in observed behaviour might occur. For instance measured migration speeds might be underestimated or the length of travelled distances might be less. It is recommended to collect independent data from more conventional methods like monitoring downstream migrating eels or tag recapture experiments besides a field telemetry experiment to examine eventual aberrant behaviours related to the used methods.

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AQUATIC TELEMETRY

ADVANCES AND APPLICATIONS

Proceedings of the
Fifth Conference on Fish Telemetry held in Europe
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