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BRIEF COMMUNICATION

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Survival, growth and tag retention of juvenile European eel (*Anguilla anguilla* L.) with implanted 12 mm passive integrated transponder tags and acoustic tags

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Abstract

To evaluate the efficiency of tagging juvenile European eels with implanted 12 mm passive integrated transponder (PIT) tags or Eel/Lamprey acoustic transmitters (ELATs), the authors studied tag retention, survival and growth of eels (7–25 g). Experimental eels were obtained from an eel farm, tagged and then released in a series of shallow dug-out ponds with a surface area of *c.* 200 m^2. Tagged and control eels were distributed evenly, with 50 tagged and 50 control eels in each of four ponds, giving a total of 200 tagged and 200 control eels mixed. After 76 days, the ponds were drained, and eels were sampled and measured. A total of 344 eels (86%) were recaptured, indicating high survival. Tag retention was 99% as only one of the recaptured PIT-tagged eels had lost the tag and none of the ELAT tagged. The results demonstrated that tagging juvenile eels >16 cm with these small tags is indeed feasible. The growth of tagged and control fish was differentiated but generally low in length and negative in mass but did not differ between the three groups.

KEYWORDS
seminatural ponds, tag expulsion, tagging effects, telemetry

The European eel population has been declining since early 1980s, and the current recruitment of glass eel is 1.2%–8.4% of the 1960–1979 reference level (ICES, 2021). To aid recovery of the eel stock, the European Council adopted a framework regulation (European Comission, 2007). Each European Union member state must issue a national eel management plan with the objective to ensure the escapement to the sea of at least 40% of the silver eel biomass relative to the best estimate of escapement that would have existed if no anthropogenic influences had impacted the stock. Thus, data on the survival of eels, from glass to silver stage, is very important for management measures (Moriarty & Dekker, 1997). Telemetry (Hussey *et al*., 2015) offers a variety of electronic tags that can provide information on different scales. For eels, most tag types [radio, acoustic, satellite (PSAT)] have been applied on adult, large silver eels, but for juvenile eels, their small body size limits the options. Small eels have been successfully tagged with visible implant elastomers (VIE) (Simon & Dörner, 2011) and coded wire tags (CWTs) (Thomassen *et al*., 2000; Jepsen *et al*. 2010). Griffioen *et al*. (2022) found that marking glass eels with VIE did not lead to increased predation. Although these methods work well, their use is limited to situations where the eels are recaptured. In most instances, these methods are used for "batch-marking," and thus, individual information/recognition is only possible upon dissection. One way to study eel survival in the field is to tag eels with passive integrated transponder (PIT) tags, allowing individual identification of live eels on recapture, by scanning (predator) habitats with a ground scanner or as registration by fixed in-stream PIT stations. Tagging small eels with 12 mm PIT tags or similar-sized juvenile Eel/Lamprey acoustic transmitters (ELAT; Deng *et al*., 2021) is a way to gain crucial new information about eel movement and behaviour. Studies focusing on predation of eel (Jepsen *et al*., 2010) and survival of stocked eels in streams (passing a PIT station on the way out) are just examples where the use of PIT tags is obvious. Eels or lampreys tagged with ELAT (Mueller *et al*., 2017) can provide solid documentation of passage efficiency/mortality at dams or other similar bottlenecks. Before using the method in the field, it is necessary to test for adverse effects of tagging and to evaluate tag retention for a longer period. Thus, the aim of this study was to estimate tag retention, survival and growth of eels as well as to compare retention of customary PIT tags with that of ELAT tags under semi-natural conditions.

Ponds of approximately 200 m^2 (192–204 m^2) were renovated during spring 2011. Vegetation and soft sediment at the bottom and sides of each pond were removed. The bottom of the ponds was formed with an inclination towards the pond outlet to facilitate efficient draining. A water pipe supplied constant groundwater flow to the ponds. The water level in the ponds was regulated by a monk sluice to keep a stable water depth of 0.9–1.1 m. Nets of 3 mm mesh-size covered the outlet pipe at the monk to prevent eels to escape. Water temperature in the ponds was measured using a temperature logger. The ponds swiftly developed into semi-natural systems with a diverse macrophyte community as well as a rich invertebrate and amphibian fauna. No food was supplied during the experiments. The ponds were open to potential predators, and grey heron (*Ardea cinerea*) were observed in the vicinity of the ponds. For a more detailed description of the experimental set-up, see Pedersen *et al*. (2017).

The experimental eels were purchased from a commercial eel farm in Denmark that imports glass eels from France and rears the eels to a size for human consumption and provides on-grown eels for restocking programmes. All eels used for this study were captured as glass eels during the previous winter and thus were a maximum of 8 months old when tagged and released.

On 30 and 31 August, a total of 400 juvenile eels were transported from an eel farm to the ponds, where they were divided into three groups: (a) control, (b) PIT tagged and (c) ELAT tagged. In two ponds, 50 ELAT and 50 controls were released, and in two ponds 50 PIT and 50 controls were stocked.

All eels were anaesthetised in a bath of 100 mg l^{-1} benzocaine (Sigma Chemical Co., St Louis, MO, USA), measured (nearest mm, total length) and weighed to the nearest 0.1 g. One hundred eels had a 12 mm half-duplex PIT tag (2.12 mm diameter, 0.1 g: Oregon RFID), and 100 eels had an ELAT (12 mm length $\times$ 2 mm diameter, 0.08 g) inserted through a 2–3 mm mid-ventral incision. The incision was made by a pointed scalpel blade, and tags were inserted by hand. Tagged eels recovered from anaesthesia within 1–3 min and batches of tagged and control eels were released into the ponds within ½ h of tagging. All control eels had a small piece of the most anterior caudal fin removed to facilitate recognition upon recapture. The non-functioning ELAT housed a full-duplex PIT tag (Biomark HPT-8, Boise, ID) for individual eel identification. Tag/-body-mass-ratios ranged from 0.3% to 1.3% (mean 0.5).

All ponds were drained on 16 and 17 November, 76 days after stocking. When a pond was drained through the outlet pipe, a bag-net (3 mm mesh-size) was used to intercept eels escaping through the outlet water. When the water depth in the pond was <20 cm, electrofishing was applied several times. Despite the intensive electrofishing, which was continued until all eels were assumed captured, the authors found that emptying a pond completely was indeed very difficult or impossible. Thus, the recapture of X% of the stocked eels does not mean that there was a mortality of 100%–X%. "Missing" eels might have been predated, left the pond or (most likely) avoided capture.

After draining the ponds, all captured eels were brought to the lab alive. At the lab the eels were euthanized by an overdose anaesthesia (500 mg l^{-1} benzocaine) and processed right away. Euthanized eels were measured (total length) and weighed to nearest millimetre and 0.1 g. There was no direct evidence of tag expulsion, but in 26 (14%) of the recaptured, tagged eels, a small bulge or mark at the abdomen was noted (Figure 1), revealing the tag position and possibly an indication of a tissue reaction leading to expulsion. To search for lost PIT tags, all four ponds were thoroughly scanned with a ground PIT scanner, and 11 tags were detected – 10 were in living eels, and one loose tag was recovered. Only regular PIT tags Half Duplex (HDX) could be detected, so no ELAT tags Full Duplex (FDX) could be recovered this way.

Potential effects of treatment and ponds on recapture rates were checked *via* χ^2 tests. Calculation of individual growth was possible only for tagged eels due to individual recognition. Nonetheless, because all eels had the same starting condition at the date of tagging, group mean length and mass at date of recapture were an appropriate proxy for testing the potential effect of tagging the eels. The possible effect of tagging on change in length and mass was tested by one-way ANOVA, whereas a comparison of non-normal distributed individual growth data of tagged eels was done by Mann–Whitney test. Differences in length and mass of eels with marks on their stomach (Figure 1) compared to eels without marks and between the treatments were tested *via* two-way ANOVA. For conducting the ANOVAs, all eel length data were square root – transformed due to left skew. Normality checks and Bartlett or Levene's test verified that the assumptions of parametric tests were met. The data analysis was performed using R 4.1.0 (R Core Team, 2021) with pooled pond replicate data.

The care and use of experimental animals in this study complied with Danish animal welfare laws, guidelines and policies as approved by the Danish National Experimental Animal Board under permission number 2017-15-0201-01164.

The ponds maintained a stable water level during the study period, and no incidents of flooding or lacking water supply occurred. The temperature varied from 4 to 14°C, the inflow of cool groundwater keeping the temperature within this range. Flora and fauna developed quickly in the ponds after initial clean-up, and plenty of natural food was apparently available for the eels during the experiments.

FIGURE 1 Examples of eels with sign of reaction (below) and no signs (above), 76 days after tagging

Of the 400 stocked eels, a total of 344 (86%) were recaptured at the termination of the experiment 75 days after tagging. The mean recapture rate was 88% for control eels, 78% for eels with ELAT and 97% for PIT tagged eels (Figure 2). Recapture rates did significantly differ between the treatments ($X^2_2 = 16.709$, $P < 0.001$) but not between ponds ($X^2_3 = 7.576$, $P = 0.056$). All the recaptured eels were apparently in good condition, and the post-mortem examination of the incisions showed only well-healed wounds and no signs of infection or necrosis. A number of tagged eels displayed a mark/discolouration of the incision area (Figure 1). This was interpreted as an indication of tissue reaction, maybe leading to expulsion of the tag at a later stage. All eels, but one, without a fin-cut still had the tag, so tag retention was 99%. The one eel that lost the tag was 23 cm and 22.7 g at tagging with a regular PIT tag.

All eels grew only little during the 75 days, and there was even a general negative growth in weight (Table 1). Neither PIT tags nor ELATs had an effect on the change in length or mass over the study

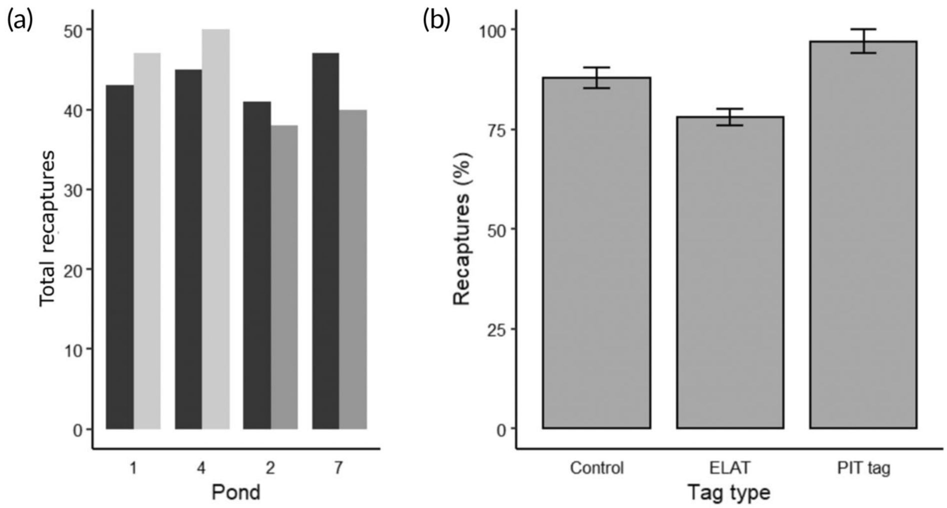

FIGURE 2 (a) Number of recaptured eels per tag type and pond as well as (b) mean recapture rates ± S. E. of different tag types for pooled pond data. ■ Control. ■ ELAT. ■ PIT tag

TABLE 1 Mean ± standard deviation (S.D.) and range of body length (cm) and mass (g) of eels measured at date of tagging (NT = number of individuals marked) and recaptured (NR = number of individuals recaptured) for different groups

	Tagging				
		Length (cm)		Mass (g)	
Type	N_T	mean ± S.D.	range	mean ± S.D.	Range
Control	200	21.9 ± 1.9	16–25.8	18.3 ± 4.5	7.9–27.9
PIT	100	21.6 ± 1.9	16.6–25.1	17.4 ± 4.1	7.5–27.3
ELAT	100	22.0 ± 1.9	16.7–25.2	18.2 ± 4.4	7.3–27.5

		Recapture				Growth	
		Length (cm)		Mass (g)		Length (cm)	Mass (g)
Type	N_T	mean ± S.D.	Range	mean ± S.D.	Range	mean ± S.D.	mean ± S.D.
Control	176	21.8 ± 2.0	15.8–26.6	17.1 ± 4.5	6.7–30.7		
PIT	97	21.8 ± 1.9	16.9–25.7	17.2 ± 4.1	6.8–28.7	0.2 ± 0.4	−0.8 ± 1.6
ELAT	78	22.2 ± 2.0	16.7–26.4	16.7 ± 4.3	8.3–27.0	0.1 ± 0.4	−1.1 ± 2.0

Note. Mean ± S.E. of individual growth of eels can only be calculated for recaptured tagged eels due to individual recognition.

Date/variable	Eel body length			Eel body mass		
	df	*F*	*P*-value	*df*	*F*	*P*-value
Tagging						
Tag type	2	1.839	0.160	2	1.592	0.205
Marks	1	8.132	<0.01	1	9.739	<0.01
Tag type: Marks	1	0.508	0.476	1	0.760	0.384
Residuals	394			393		
Recapture						
Tag type	2	1.188	0.306	2	0.437	0.646
Marks	1	11.245	<0.001	1	12.533	<0.001
Tag type: Marks	1	0.613	0.434	1	0.264	0.608
Residuals	346			346		

TABLE 2 Results of two-way ANOVAs, analysing differences in length or mass of the eels between tag types (PIT tag or ELAT) and eels showing marks compared to eels without marks at the date of tagging and recapture

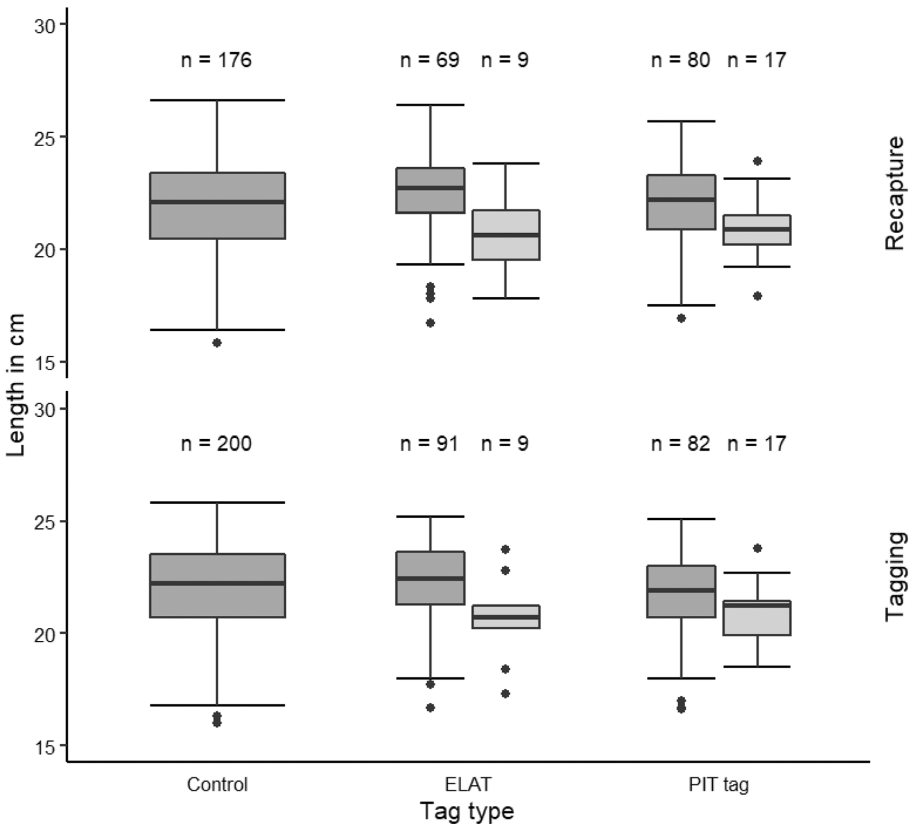

FIGURE 3 Eel body length (cm) at tagging and at recapture of fish displaying signs of tissue reaction compared with the length of fish showing no indication of this at recapture. ▭ no marks at recapture. ▭ showed marks at recapture

period (length: $F_{2,348} = 1.272$, $P = 0.282$; mass: $F_{2,348} = 0.31$, $P = 0.734$). The 26 eels that showed marks as sign of beginning expulsion at recapture date were significantly smaller at tagging as well as at recapture than other eels – independent from tag type (Table 2; Figure 3). Nonetheless, individual growth of these eels showing marks at recapture date was not affected by either of both tag types – regarding neither length nor mass (Table 3). Eels that disappeared were similar to the rest in length and mass at date of tagging (mean length = 21.6 ± 0.4 cm, mean mass = 17.3 ± 1 g; compare Table 1).

Despite the open character of the ponds, where predators could prey on the stocked eels, the PIT-tagged eels obviously thrived and had a high relative survival (97%) comparable with that of the control fish (88%). The ELAT-tagged eels had a slightly lower relative survival (78%). With only one tag loss, overall tag retention was unexpectedly high. Thus, a lab test of similar ELAT dummy tags in American eel (*Anguilla rostrata* Lesueur) showed much lower tag retention with 50% tag loss after just 26 days (Mueller *et al.*, 2017). The reason for this may be found in the fact that the tag/body mass ratio was significantly higher (1.1%–3.6%.) in the rostrata study; that is, the eels were smaller than in this study (tag/body mass <1.3%). This is a phenomenon often observed (*e.g.*, Wargo-Rub *et al.*, 2014) where the risk of tag expulsion is highly increased with increased

TABLE 3 Results of Mann–Whitney U (Wilcoxon rank sum) tests comparing growth (length and mass) of eels with marks *vs.* without marks between the tag types (PIT tag and ELAT)

			Eel body length growth		Eel body mass growth	
Treatment	n_{marked}	n_{others}	*W*	*P*-value	*W*	*P*-value
ELAT	9	69	277.5	0.609	375.5	0.313
PIT	17	80	505.5	0.096	555.5	0.239

tag/body mass ratio. The fact that the authors observed possible indication of beginning expulsion in many of the smallest recaptured eels (Figures 1 and 3) also supports the conclusion that the authors were close to the lower size limit for tagging eels with tags of this size.

The relative survival/capture rate for ELAT-tagged eels was significantly lower than that of PIT-tagged, indicating that more of these died during the study. This is due to the fact that the authors could search and find eels tagged with HDX-PIT tags but not the ELAT tags (with FDX-PIT tags) with the ground scanner. If the tagged eels recovered by scanning (total of 10) are removed from the analyses, the recapture/relative survival does not differ between treatments.

In other studies, a reduced growth of tagged fish has been reported (*e.g.*, Cooke *et al*., 2010; Jepsen *et al*., 2008). In this study, however, there was no indication of a reduced growth of tagged eels compared with controls. Similar high retention and survival and unaffected growth were found for yellow shortfin eels (*Anguilla australis*) in a 108 days' lab trial (Hirt-Chabbert & Young, 2012). On the contrary, Mazel *et al*. (2013) tested the effect of PIT tagging on growth of yellow European eels in the field and found significantly reduced growth of tagged eels compared to untagged (estimated from otolith readings), 1–11 years post-capture. The size of eels at tagging in the Mazel study was similar to this study, with eels of 20–30 cm tagged with 12 mm PIT tags. It is, however, difficult to compare growth in eels where only a small sub-sample is used, because of highly differentiated growth observed in wild eels (*e.g.*, Melia *et al*., 2006).

The fact that growth in this study was very limited as 18% of the tagged eels had negative length growth and 85% lost weight is not surprising, considering that these eels were transferred from a rearing facility (excess food and ideal temperature) to the natural conditions with a limited food supply and varying temperatures. This is likely the reason for the observed overall positive length increment and negative mass increment (Table 1), and the loss of mass in stocked on-grown eels has been described in detail by Simon *et al*. (2013) and Pedersen (2009). Growth was highly differentiated in all three treatment groups (Table 1), and some individuals gained up to 6 g, whereas others lost up to 7 g during the 76 days. It was clear from the results that carrying a tag does not significantly reduce growth. Nonetheless, in the case of such low growth as seen here, a comparison of growth is not a very good indicator of sublethal tagging effects. Some studies have indicated that a reduced feeding/growth may be caused by the simple fact that an implanted object takes up a significant part of the body cavity, thus reducing appetite (Chrysafi *et al*., 2021), but the data here show no difference in growth between tagged and control fish.

The authors acknowledge that testing tagging effects under field or semi-natural conditions like in this study does pose risks and challenges not encountered in a controlled lab environment. Nonetheless, they do believe that the results of such studies better fulfil the aim, namely to test the usefulness of a specific approach (tag/method/species/size) for field studies (Jepsen *et al*., 2008).

The results clearly demonstrate that even for relatively long-term field studies, tagging of juvenile eels in the size range 16–25 cm with 12 mm PIT tags or ELATs is feasible and that very little tag loss or mortality should be expected.

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DATA AVAILABILITY STATEMENT

None of the data used here have been shared, so all data are available and can be requested from the author.

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