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Short communication

Tagging juvenile seabass and sole with telemetry transmitters: medium-term effects on growth

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The effects of tagging with acoustic transmitters on the growth of juvenile seabass, *Dicentrarchus labrax* (L.) (initial mean mass ± SD, 173 g ± 23.4) in a 47 d tank experiment, and sole, *Solea solea* (L.) (103.2 g ± 14.8) in a 72 d tank experiment and (104.0 g ± 18.4) in a 58 d salt marsh mesocosm experiment were examined. Twenty externally tagged seabass grew more slowly than the 20 with surgically implanted tags, which reached the same mass as nine control fish. Movements of the external transmitter's harness caused abrasions of the skin and loss of the tag in 60% of the cases. We thus recommend implanting transmitters for telemetry studies of juvenile seabass weighing between 120 and 214 g and carrying a tag that represents 2.2–2.5% of body mass. Both tank and mesocosm experiments conducted on juvenile sole concluded that the externally attached tag retention rate was good, but at the expense of the fish growth rate.

Keywords: adaptation, juvenile fish, marking techniques, telemetry.

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Introduction

A large number of field and laboratory studies have demonstrated the usefulness of radio and ultrasonic telemetry in a wide range of applications (Baras, 1991). However, the transmitter attachment may influence the behaviour and performance of the fish (Lewis and Muntz, 1984; Mellas and Haynes, 1985). These potentially negative effects remain relatively poorly studied for a number of species and tagging methods.

Independent of the tagging method, tagged fish must initiate various adjustments in order to recover hydrostatic equilibrium and/or maintain neutral buoyancy in the water column because of the additional load. Compensation through active swimming is energetically expensive (Lefrançois *et al.*, 2001) and probably results in redistributing the fish's energy balance, at the cost of swimming performance, somatic or gonadal growth. This is particularly the case when an external tag is used because of the extra drag forces it induces which the fish must compensate for. This paper presents experiments on the medium-term effects of tagging on growth of juvenile seabass (*Dicentrarchus labrax* (L.)) and juvenile sole (*Solea solea* (L.)).

Materials and methods

Seabass experiment

In late August 2000, 49 individually pit-tagged seabass (20 months old) originating from a fish farm (Extramer, France) were placed in a 1 m^3 circular tank equipped with a demand feeder at constant water temperature (21.8°C) and salinity (39.5). On 21 September (day 0, D0), the fish were weighed (average mass ± standard deviation: 173 g ± 23.4) and partitioned into three groups of homogeneous mass distribution: a control group (CG), containing nine fish and two groups of 20 fish each which were tagged with dummy tags (V8SC Vemco Ltd, cylindrical 9 mm × 24 mm, 4.2 g in air) using either external (EG) or intra-peritoneal (IG) tagging methods (Winter, 1983).

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Before tagging, the fish were anaesthetised in a solution of 0.5 ml l^{-1} 2-phenoxy-ethanol and an antibiotic added to the anaesthetising solution until loss of equilibrium was observed. External tagging was accomplished by passing two kevlar sutures 1 cm apart through the muscle underneath the pterigiophores of the dorsal fin and a smooth plastic plate was placed on the opposite side where threads were knotted and glued with instant glue. Surgery for internal tagging was done with a 1 cm opening through the ventral muscle in front of the pelvic fin, the tag was pushed gently inside the peritoneal cavity, then two sutures were made with polyamide monofilament to close the wound. Tagging took about 2 min, a local antiseptic solution (Betadine®, iodised polyvidone) was applied to the wounds and the fish were transferred back into clean water. The fish were held with the mouth at the water surface until active swimming was recovered. The tank was under constant video surveillance. Demand feeding activity of the group was recorded as the seabass manipulated a rod that delivered food.

On 10 October (day 26, D26), all the fish were weighed and observations made on the external lesion and healing of the tagging wound. The study was ended on 7 November (day 47, D47), when the fish were deeply anaesthetised until death, weighed, examined for external lesion and healing of the tagging wound, dissected for intra-abdominal examination of the area surrounding the dummy tag, and for sex determination.

Sole experiments

Two experiments were conducted with juvenile soles: the first using tanks, the second in a pond mesocosm on a salt marsh. For the tank experiment, soles were captured in the "pertuis Breton", north of La Rochelle (46°15′N, 01°06′W) by a traditional trawler "Le Rescator" on 22 September 2001 and transferred to PVC tanks (1 m^2 in surface and 0.8 m^3 in volume), with a bottom sand layer and an open circuit pumping water from a nearby salt marsh. On 3 October (Day 0 for tank experiments, $D0_t$), they were weighed (average mass ± SD: 103.2 g ± 14.8), measured for standard length (average length ± SD: 20.8 cm ± 1.1) and individually tagged under light anaesthesia (2-phenoxy-ethanol 0.2 ml l^{-1}) with a visible implant (VI Alpha). The fish were divided into two groups of homogeneous size class distribution of 18 individuals each: one control group and one externally tagged group. Dummy tags (V8SC Vemco Ltd, cylindrical 9 mm × 20 mm, 3.3 g in air) were externally attached on the eyed face underneath the pterigiophores of the dorsal fin (median region), similar suturing methods as for the seabass were used. Antiseptic solutions (Betadine® and Polypra®) were applied at the point of sutures.

Fish from both the control and tagged groups were mixed and randomly assigned to one of three experimental tanks. Growth (mass and length measurements) and health status were checked fortnightly (18 and 31 October, 16 and 30 November). During the 72 d of the experiment, the fish were fed every 2 to 3 d with fresh mussels at a ratio of 2% of the biomass. The tank experiment with the soles was ended on 14 December ($D72_t$) following the same procedure as for the seabass experiment.

Left sagittal otoliths were extracted and prepared for age determination. Transverse sections of the otoliths were sampled using standard techniques (see Secor *et al.*, 1992) and examined with light microscopy, before and after staining. In unstained slides, the age estimation was based on the number of opaque zones (Vianet *et al.*, 1989). Staining with Toluidine Blue solution (0.5% in a 2% acetic acid solution) was used as necessary to enhance annuli deposited in the innermost part of the opaque zones (Lagardère, unpublished data).

The second experiment using soles took place from 27 March to 24 May 2002, this time using a salt marsh pond as a mesocosm. Soles, captured in the "pertuis Breton" by trawling (28 February and 1 March) by the fishing boat "Le Rescator", were maintained in similar tanks and fed with similar procedures as in the tank experiment. On 27 March (Day 0 for marsh experiments, $D0_m$), 18 individuals of homogeneous size were weighed (average mass ± SD: 104.0 g ± 18.4) and measured for standard length (average length ± SD: 20.7 cm ± 0.9), and tagged with a visible implant (VI Alpha) under light anaesthesia (2-phenoxy-ethanol 0.2 ml l^{-1}). The fish were then divided into two homogeneous groups: one control group and one externally tagged group using the same tagging technique as described in the previous section. After a recovery period in the same tanks, the soles were released into the experimental earthen pond on 30 March. While the fish were in the pond, they fed on whatever natural prey were present in the pond. The fish were recaptured on 24 May, 58 d after tagging ($D58_m$), and the experiment ended as described in the previous section.

Data analysis

Non-parametric tests (Kruskal–Wallis one-way analysis of variance) evaluated the effects of tagging on fish growth (Scherrer, 1984). Individual fish lengths and masses were the test variables and the factors were either tank number, fish sex, tagging treatments (control, externally or internally tagged) or the dates on which the measurements were taken. Statistical analyses were performed with Systat® 7.0 for Windows (SPSS Inc.). Specific growth rates (SGR) were calculated using the formula $SGR\ (\%/day) = 100\ [(\ln(M_2) - \ln(M_1))/(t_2 - t_1)]$, where M_2 is average mass at time t_2, and M_1 is average mass at time t_1 (after Alänära, 1992).

Results

Effects on juvenile seabass growth

No mortality was observed among the seabass, and feed demand and intake returned to the values recorded in the

3 weeks prior to manipulation within a day of the tagging procedure. The daily feeding rate of the group calculated from D0 to D47 was 1.14, for a conversion factor of 1.66. The video survey indicated no apparent posture changes between the treatment groups, and the equilibrium of EG fish did not change appreciably.

No significant difference in mass was found among sexes at the start of the experiment (at D0, KW, $P = 0.56$, $N = 49$, $df = 1$) or later on between treatment groups (CG: 1 female of 9, EG: 3 females of 20, IG: 2 females of 20). Therefore, sex was not taken into account as a factor for further analysis. The growth increase was similar for both the control (CG) and intra-peritoneal groups (IG) (Figure 1), but was slower for the external group (EG). There was no difference in body mass at D0 among groups, and no difference between the CG and IG groups at D26 and D47 (Table 1). IG fish showed an SGR of 0.76% from D0 to D47 whereas the CG fish had an SGR of 0.89%. Over the same time period, the SGR of EG fish only reached 0.47%. In all three groups, the fish grew significantly (Table 1).

The healing process was rated as very satisfactory in 19 IG fish of 20 at D47 (six fish showing a complete healing with no suture left); dummy tags were coated with visceral tissue and even attached to body wall muscle. Among the EG seabass, 12 had lost their tag harness by D47 (two had lost it already by D26) and these fish had a significantly higher mass at D47 than those eight fish still carrying tags (KW, $P < 0.01$, $N = 20$, $df = 1$). Fish that had lost their tag harness gained mass (Figure 1) fast enough to recover masses similar to the CG and IG fish on D47 (KW, $P = 0.24$, $N = 41$, $df = 2$). Tag loss was mainly a result of sutures cutting through the dorsal musculature. Fish that were still tagged at D47 showed scale loss and inflammation at the harness site, whereas fish that had lost their tag had minimal inflammation or even complete healing.

Effects on juvenile sole growth

The autumn individuals used for the tank experiment were 1 year old. This allowed us to estimate that the spring samples used in the mesocosm experiment were, at least for the most part, 2 years old. The only confirmed mortality was one control fish in the tank experiment. In the salt marsh experiment, two individuals were not recaptured (one control and one tagged fish); they might have either escaped outside the pond enclosure or died.

For the tank experiment, no differences in fish mass were found among tanks (KW at $D0_t$, $P = 0.88$, $N = 36$, $df = 2$; at $D72_t$, $P = 0.79$, $N = 35$, $df = 2$). There were no significant differences in initial masses between sexes at the start of the experiment (at $D0_t$, KW, $P = 0.69$, $N = 35$, $df = 1$), or later on within treatment groups (control fish: 12 females of 17, tagged fish: 10 females of 18). Therefore the data were pooled and neither tank number nor sex was taken into account for further analyses. Water temperature showed large fluctuations, and decreased from 20°C down to near zero during December (Figure 2).

A significant difference in mass appeared at $D27_t$ after tagging, and continued to increase until the end of the experiment (Figure 2, Table 2). Such differences were not observed for standard lengths (Table 2). Comparing final and initial mass values for control fish showed that both masses and standard lengths increased: fish grew by an average of 27.4 g and 9 mm (SGR = 0.33%). For the tagged group, mass and standard length were similar, and the fish did not grow significantly, nor did they lose mass;

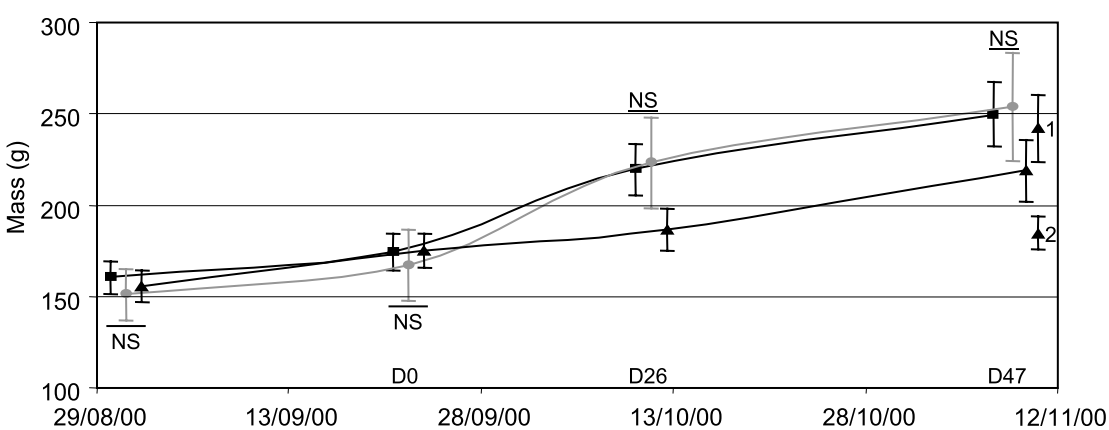

Figure 1. Averaged seabass mass (± confidence interval) for the control group (CG: grey circles), internally tagged group (IG: black squares) and externally tagged group (EG: black triangles) on different dates. Numbered black triangles correspond to 1: average mass value for fish that had lost their harness by D47 ($N = 12$), 2: average mass value for fish still carrying a tag ($N = 8$). Tagging occurred on day 0 (21 September 2000, D0) and mass measurements were taken on day 26 (10 October 2000, D26) and on day 47 (7 November 2000, D47). NS stands for non-significant Kruskall–Wallis test results (Table 1).

Table 1. Results of Kruskall–Wallis test (KW: probability, df: degrees of freedom) comparing fish (seabass) individual wet mass between treatments (CG: control group, IG: internally tagged group, EG: externally tagged group) on different measurement dates (D0, D26, D47).

Test		KW P	N	df
Comparing mass of all three groups on:	D0	0.52	49	2
	D26	<0.01	49	2
	D47	0.04	49	2
Comparing mass of CG and IG on:	D0	0.29	29	1
	D26	0.89	29	1
	D47	0.83	29	1
Comparing initial and final mass for:	CG	<0.01	9	1
	IG	<0.01	20	1
	EG	<0.01	20	1

the SGR was 0.08% (Table 2). Although not statistically significant, it can be noted that for four fishes out of 10, and two fishes out of eight, a mass loss was observed for tag to fish mass ratios ≥3 and <3%, respectively.

For the mesocosm experiment, water temperature in the pond followed natural seasonal patterns: 10°C at the start of the experiment in March and rising to 20°C toward the end of May (Figure 2). There were no significant differences in initial mass nor length between treatment groups (Table 2).

A significant difference between treatment groups appeared for both mass and standard length at $D58_m$ (Figure 2, Table 2). For both groups, fish did not grow significantly nor did they lose mass (Table 2) even if control fish had an SGR of 0.28% and tagged fish an SGR of −0.07%. Although not statistically significant, it can be noted that all sole with tag to fish mass ratio ≥2.9 and ≤3.7% lost mass, and one fish with a tag to fish ratio of 2.8% maintained its mass.

For both experiments, it appeared that all sole were in apparent good condition, correctly pigmented and without visible external infection. Tagged sole showed epidermal erosion at the point of contact with a deeper erosion ahead of the tag, although no inflammation of the derm was observed.

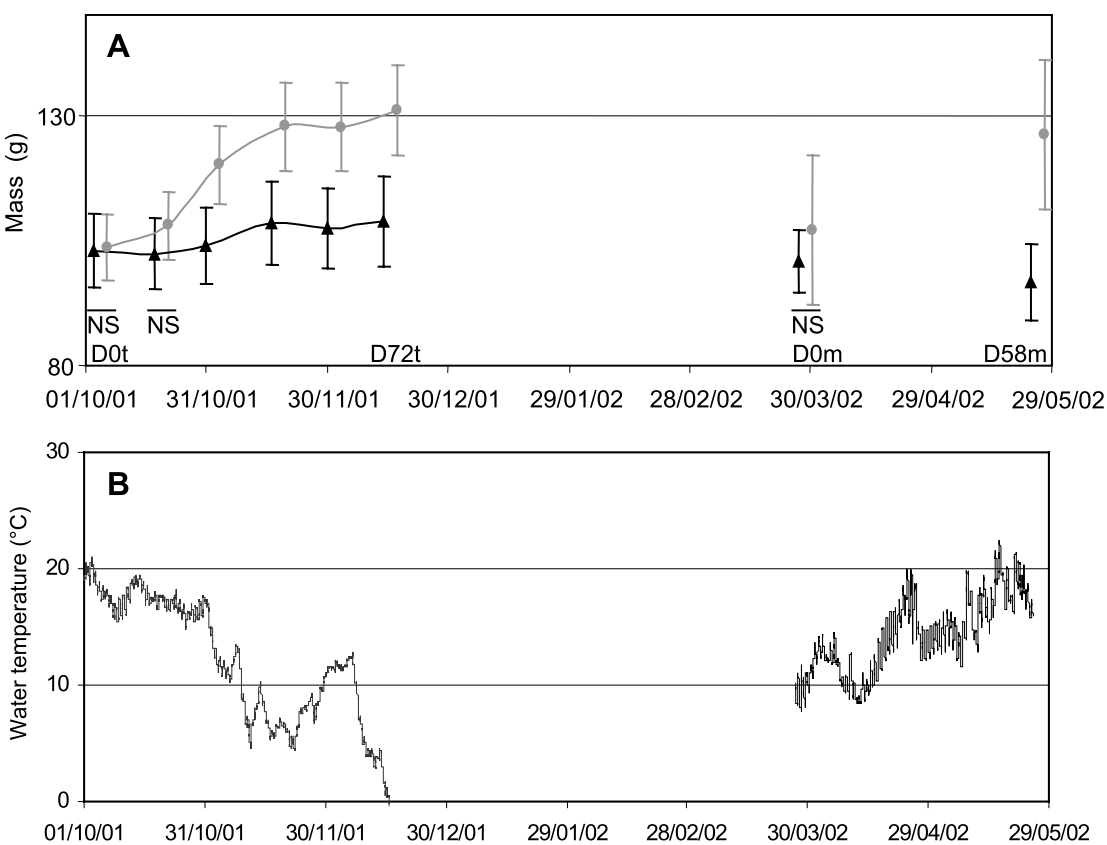

Figure 2. (A) Averaged sole mass (± confidence interval) for the control group (grey circles) and externally tagged group (black triangles) over time. For the tank experiment, tagging occurred on 3 October 2001 ($D0_t$) and mass measurements were taken fortnightly until 14 December 2001 ($D72_t$). For the salt marsh experiment, tagging occurred on 27 March 2002 ($D0_m$) and the experiment ended on 24 May 2002 ($D58_m$). (B) Water temperature during the experiments. NS stands for non-significant Kruskall–Wallis test results (Table 2).

Table 2. Results of Kruskall–Wallis test (KW: probability, df: degrees of freedom) comparing fish (sole) standard lengths (SL) and wet mass (M) between treatments on different measurement dates.

	Date	Variable	KW P	N	df
Tank experiment					
Comparing control and tagged fish:	3 Oct. $D0_t$	SL	0.74	36	1
		M	0.96	36	1
	18 Oct.	SL	0.70	36	1
		M	0.38	36	1
	31 Oct.	SL	0.22	35	1
		M	0.03	35	1
	16 Nov.	SL	0.15	35	1
		M	<0.01	35	1
	30 Nov.	SL	0.12	35	1
		M	<0.01	35	1
	14 Dec. $D72_t$	SL	0.12	35	1
		M	<0.01	35	1
Comparing initial and final values for control fish:		SL	0.04	35	1
		M	<0.01	35	1
Comparing initial and final values for tagged fish:		SL	0.61	36	1
		M	0.27	36	1
Salt marsh experiment					
Comparing control and tagged fish:	$D0_m$	SL	0.39	19	1
		M	1.00	19	1
	$D58_m$	SL	0.01	16	1
		M	<0.01	16	1
Comparing initial and final values for control fish:		SL	0.07	16	1
		M	0.09	16	1
Comparing initial and final values for tagged fish:		SL	0.46	16	1
		M	0.25	16	1

Discussion

Our results showed that juvenile seabass could survive and grow with either an external or surgically implanted transmitter. In our study set-up, we were unable to detect inter-group differences in feed intake. However, the groups' ability to consume food was not affected by any of the tagging manipulations, feed demand returned to previous levels within a day and the video observation did not reveal any abnormal behaviour. The absence of effect on feeding activity by tagging has already been demonstrated by other investigators (Mellas and Haynes, 1985; Lucas, 1989; Moore *et al.*, 1990; Moser *et al.*, 1990; Knights and Lasee, 1996), however, the results of this study suggest that the fish's ability to grow was affected less by surgically implanted transmitters than by the external tagging method. As telemetry data are often used to make inferences about an entire population, the method of transmitter attachment should be the one that least affects the studied animal. The near normal growth of fish with surgical implants indicated that this method might be more suitable for monitoring the movements of juvenile seabass. In contrast, the reduced growth rate of externally tagged fish may in turn affect behaviour and result in telemetry data that are not representative of the movements of the studied population.

Masses never differed significantly between control fish and seabass that were operated on, indicating that the surgical procedure and presence of the tag did not affect growth rates when the average initial tag to fish (mass in air) ratio was 2.2%. Others have reported that surgical procedures did not affect medium-term growth (Mellas and Haynes, 1985; Lucas, 1989; Moore *et al.*, 1990) and that fish with surgical implants had food in their stomachs (Mortensen, 1990). In contrast, the growth rates of externally tagged fish were significantly less than the other groups suggesting that external tagging, using an average initial tag to fish weight ratio of 2.5%, affected fish performance, through both the tagging procedure and the additional load which requires extra energy expenditure dedicated to swimming instead of growth. Lewis and Muntz (1984) showed that externally attached radio transmitters raised both the tail beat frequency and opercular beat rate of juvenile rainbow trout. These variables are both indicative of how much energy fish are expending (Bainbridge, 1958; Shepherd, 1973) and thus, we hypothesised that externally tagged fish devote more energy to swimming activity and that less energy would then be available for growth. This is consistent with our observations that fish, which lost their tag harness, subsequently regained mass (within 21 days); this recuperation suggested the growth rate potential of the seabass was being impaired by the burden of the external tag. As all externally tagged fish grew significantly through the test period, we can infer that their food intake was likely to be normal, but their energy expenditure was diverted from growth processes.

Chronic and multiple acute forms of stress have been shown to adversely affect fish physiology (Pickering *et al.*, 1982) and behaviour (Sigismondi and Weber, 1988). In addition, incisions made during surgery may become infected (Lucas, 1989; Knights and Lasee, 1996). However, once fish have recovered, surgical implantation does not cause significant effects on growth, and proper surgical procedures can reduce or eliminate the incidence of infections. Furthermore, considering the loss rate of tags associated with external tagging, we recommend surgical implantation for telemetry studies of juvenile seabass weighing between 120 and 214 g.

The experiment conducted with juvenile sole showed that externally tagged fish can survive, grow and retain the tag for at least 72 days in tanks and 58 days in a salt marsh mesocosm. However, the fish's ability to grow was affected by external tagging in both experiments. The lack of comparable studies on flatfishes renders a comparison difficult. Only Szedlmayer and Able (1993) reported on the

effect of external tagging on summer flounder growth in length. They showed an 8 mm growth obtained over a 94 d laboratory period for two fish (246 and 272 mm TL) tagged with a 4 g (in air) ultrasonic transmitter. This value is comparable with the 9 mm length increase obtained in the present study over a 72 d winter period for control fish. However, if our analysis had been based on length measurements only, we would have concluded that the increase in standard length of control and tagged fish was similar (Table 2). Whereas tagged fish did not increase significantly in mass, control fish did. Thus the mass measurement is a more sensitive index of medium-term growth than length. The tank study also emphasised the importance of an acclimation period at least 15 days before control and tagged fish growth increased again, despite cold water conditions. Thus we recommend the minimum duration of an experiment should be 1 month when designing experiments to study the effects of any treatment on juvenile sole growth.

The mesocosm salt marsh experiment further confirmed the inhibitory effects of external tagging on juvenile sole growth in mass and length. However, the performances of both groups (control and tagged) were low since no significant growth in terms of either length or mass was observed even though the experiment took place during a 58 d period under spring conditions. These poor performances, probably due to unfavourable environmental conditions, reinforced the effects of external tagging when the tag to fish mass ratio varied between 2.8 and 3.6%.

Finally, the present results on flatfish again raise the question about a maximum threshold for the tag to fish mass ratio similar to that demonstrated for round body fishes (Winter, 1983; Adams *et al.*, 1998); a limit may apply to flatfish as well. Arnold and Holford (1978) concluded that a 40 cm long tagged plaice has to increase its power output by up to 5% to maintain the same speed as an untagged fish in a study on the physical effects of acoustic tags (1 cm in diameter by 5 cm long, 8.2 g mass in air). From this study, we can estimate that a 20 cm long fish would need extra power output ranging from 5 up to 15% depending upon the fish body drag coefficient (calculated for a particular drag coefficient of the tag). Arnold and Holford (1978) attributed this extra energy expenditure to drag forces that the fish has to swim against and they considered that it was almost negligible for fishes above 40 cm in length. However, extra energy devoted to swimming activity, and possibly diverted from somatic growth, may explain the inhibitory action of the external tag on the medium-term growth of 20 cm long juvenile sole, even if our tag was smaller in size. Arnold and Holford (1978) further suggested that the drag coefficient of the tag can be significantly reduced by streamlining its shape. Based on the present study, we recommend working with a tag to fish ratio below 2%, unless the shape of the tag could be changed to a flat disc to minimise drag effects on flat fishes.

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