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TELEMETRY CASE REPORT

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Operculum PIT tagging: a viable alternative to avoid human consumption in processed salmon

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Abstract

Background: Passive integrated transponder (PIT) tags are commonly used to identify individual fish. However, use of PIT tags in commercial aquaculture research is limited by consumer safety concerns. For farmed fish, it is critical that tags do not end up in the final product. One possibility to enable the use of PIT tags in commercial research is to insert tags into a part of the body that will be separated from the trunk during processing. We compare tag loss, mortality rate and welfare scores between Atlantic salmon post-smolts ($n = 798$) marked with PIT tags either in the operculum musculature or the abdominal cavity (standard practice) before and after mechanical delousing.

Results: We found that neither condition factor (K) (range 0.60–1.99) nor tagging location significantly affected tag loss (operculum = 6%, intraperitoneal = 8%, $z = 1.46$, $p = 0.14$) or mortality (operculum = 2%, intraperitoneal = 2%, $z = 0.55$, $p = 0.58$). However, on average, the fish which died weighed 20% less at the time of handling (271 ± 13 g, $K = 1.12 \pm 0.02$) than those which survived (340 ± 3 g, $K = 1.14 \pm 0.004$), and those which lost tags (291 ± 7 g, $K = 1.11 \pm 0.02$) weighed 15% less than those which retained them (340 ± 3 g, $K = 1.14 \pm 0.004$), irrespective of tagging location or handling treatment.

Conclusions: Fish tagged in the operculum musculature had comparable rates of mortality and tag loss to the current "best practice" standard of intraperitoneal tagging. We show that placement of PIT tags in operculum musculature is a viable alternative to placement in the peritoneal cavity.

Keywords: Passive integrated transponder, Intraperitoneal, *Salmo salar*, Tag effects, Aquaculture, Fish, Welfare

Background

Because they are small (12–24 mm long), lightweight, durable and inexpensive, passive integrated transponder (PIT) tags are commonly used to identify individual fish) [13]. Typically, PIT tags are inserted into a fish's abdominal cavity either via injection [1] or through a small incision along the ventral midline (e.g., [6]. Both methods are quick and avoid the need for sutures [5]. However, when PIT tags are inserted intraperitoneally, they often migrate from their initial insertion point which makes recovery difficult and increases the possibility that tags may enter the edible fillets [10]. Therefore, despite intraperitoneal PIT insertion being the most common attachment method and generally regarded as the "best practice" concerning fish health, placing a tag in the abdominal cavity introduces significant risk to consumers [3]. As a result, despite their functionality, PIT tags are not widely used in commercial aquaculture research. Exploring alternative tag placement where the likelihood of loss and accidental human ingestion is minimized is necessary for this technology to be used in aquaculture research at commercial scale.

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Prentice and Park [10] examined the feasibility of different anatomical locations for PIT tag attachment in juvenile and adult salmonids. They observed low rates of tag loss and mortality when salmon were tagged in both the operculum musculature and abdominal cavity, but concluded that placement of tags in the abdominal cavity was preferable due to better wound healing and marginally better tag retention. For filleted fish, however, the operculum is generally removed along with the head. Therefore, the operculum musculature is a practical location for PIT tagging commercially reared fish if the high rates of tag retention and survival associated with intraperitoneal tagging can be achieved without compromising fish welfare.

We compared tag loss, mortality and welfare scores between farmed Atlantic salmon (*Salmo salar*) that were PIT tagged via surgical intraperitoneal insertion or injection into the operculum musculature after undergoing a common farm stressor in the form of mechanical delousing.

Results

Eighteen days after tagging, mortality was 2% for fish tagged in both the operculum (7 out of 350 fish died) and abdominal cavity (9 out of 448 fish died), with no significant difference between the two tagging methods (z value = 0.58, $p = 0.56$). Treatment (deloused vs procedural control) was not an important determinant of probability of tag loss or mortality, and no mortalities occurred prior to treatment. There was also no significant difference (z value = 1.46, $p = 0.14$) in tag loss between the two tagging methods (total tag loss operculum = 6%, total tag loss peritoneum = 8%). Indeed, among the explanatory variables tested (fish weight, condition factor, PIT location and treatment), only weight was an important determinant of probability of tag loss or mortality (Table 1).

Small fish were both more likely to die and lose tags than heavier fish, regardless of PIT method, treatment or condition factor (Fig. 1). On average, the fish which died weighed 20% less (271 ± 13 g) than those which survived (340 ± 3 g), while those which lost tags (291 ± 7 g) weighed 15% less than those which retained them (340 ± 3 g). Condition factor ranged from 0.60 to 1.99 and was not an important determinant of mortality (z value = 0.19, $p = 0.85$), or tag loss (z value = 1.53, $p = 0.13$), in this trial. However, fish with higher condition factor did have significantly better welfare after treatment than fish with lower condition factor (t value = − 2.05, $p = 0.04$). Neither weight, PIT location nor treatment significantly affected welfare score (Table 1).

Table 1 Estimated regression parameters, standard errors, z values (binomial), t values (gamma) and *p* values for the GLMM's of probability of death, tag loss and post-treatment welfare score as a function of weight, PIT location, condition factor (K) and treatment (deloused or procedural control)

	Estimate	Std. error	*z* value	*p* value
Mortality				
Intercept	− 3.24	3.25	−1.00	0.318
Weight	− 0.01	0.00	− 2.66	0.008*
PIT location (opercula)	0.30	0.55	0.55	0.580
K	1.73	2.80	0.62	0.537
Treatment (deliced)	1.49	1.04	1.43	0.152
Tag loss				
Intercept	− 1.00	1.14	− 0.88	0.380
Weight	− 0.01	0.00	− 3.34	0.001*
PIT location (opercula)	0.57	0.39	1.46	0.144
K	0.74	0.48	1.53	0.127
Treatment (deliced)	− 0.10	0.44	− 0.24	0.814
	Estimate	**Std. Error**	***t* value**	***p*-value**
Welfare score				
Intercept	0.58	0.09	6.82	< 0.001*
Weight	0.01	0.01	0.59	0.559
PIT location (opercula)	0.00	0.01	− 0.15	0.880
K	− 0.19	0.07	− 2.69	0.007*
Treatment (deliced)	0.02	0.03	0.51	0.612

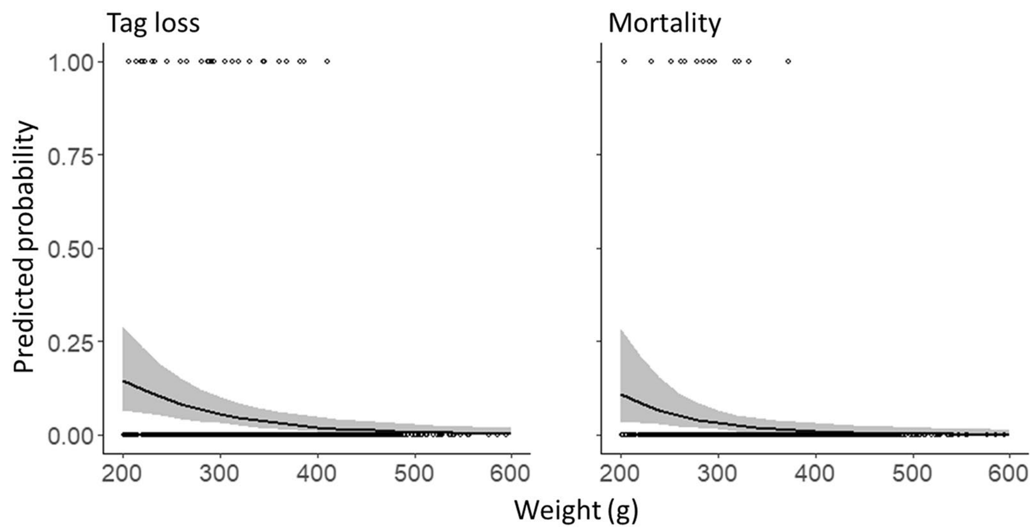

Fig. 1 Influence of weight on probability of tag loss (**a**) and mortality (**b**). Solid lines display the fitted binomial GLMM while shaded areas show the 95% confidence intervals. Points present individual observations

Discussion

Our results indicate that the operculum musculature is a viable alternative location for PIT tagging with comparable rates of tag loss and mortality to that of the widely utilized "best practice" of intraperitoneal PIT insertion. In addition, because the operculum is a small anatomical area not involved in swimming, post-mortem tag removal was faster and simpler in operculum tagged fish compared to those tagged intraperitoneally.

In Prentice and Park [10], tag retention in both the operculum and body cavity was 100% for adult Chinook salmon held in seawater over a 23-day period. However, in contrast to our results, tag retention was higher for individuals tagged in the body cavity (93%) than those tagged in the operculum (73%) over a 102-day period for juvenile Chinook salmon held in freshwater. Since our study spanned 18 days it is possible that over a longer duration similar differences in tag retention between methods would arise. However, unlike Prentice and Park [10], our study also included mechanical delousing which accelerated tag loss and mortality, and therefore likely exacerbated possible differences between tagging methods. Future research should examine the rate of mortality, tag retention and any sublethal effects of salmon PIT tagged in the operculum in the long term.

Also, despite PIT tags not exceeding 7% of fork length in this trial, well below the 17.5% limit proposed by Vollset et al. [13], mortality and tag loss after handling were significantly influenced by fish size. Two-hundred gram salmon were more likely to die and lose tags than larger individuals (400+g), likely as a result of their reduced ability to cope with stressful situations [9]. Models predict that salmon weighing 200 g or less would experience more than 10% mortality after handling compared to 2% for 300 g salmon and 0.3% for fish weighing 400 g or more (Fig. 1). For larger salmon (400+g), tag loss and mortality were negligible both before and after handling. Thus, though only suggestive due to the small number of individuals which died, the influence of weight on mortality and tag loss, irrespective of tagging location, suggests that the size standards established for internal tags in previous experiments may not be applicable in the higher stress conditions of commercial aquaculture where handling is unavoidable [7].

Commercially reared salmon are transferred from land-based tanks to sea when they are between 80 and 150 g. After sea transfer, fish are likely to experience crowding, pumping, freshwater bathing and delousing as part of regular husbandry. Here, tagged fish underwent mechanical delousing only 4 days post-tagging. Hvas et al. [4] found that salmon implanted with heart-rate monitor tags fully recovered 2–3 weeks after surgery. While PIT tagging is a less invasive procedure compared to surgical implantation of heart-rate monitor tags, 4 days may be insufficient for complete recovery. Future research on tagged farmed fish should consider how common husbandry practices can influence tagged fish welfare, survival and tag retention, and ideally allow for 2–3 weeks between tagging and exposure to handling.

Conclusions

Overall, fish tagged in the operculum musculature had comparable rates of mortality and tag loss to the current "best practice" standard of intraperitoneal tagging and represents a viable alternative for studying fish in commercial aquaculture where consumer risk is a factor. While further research is needed to understand the long-term and behavioural effects of tagging fish in the operculum musculature, our results provide a foundation from which this technology can transfer from experimental to commercial scale research.

Methods

A single cohort of Atlantic salmon (*Salmo salar*) post-smolts originating from an Aquagen strain reared at the Matre Research Station operated by the Norwegian Institute of Marine Research was divided into two groups ($n=450$ fish per group) on 11 June 2020 and placed in two separate circular tanks (diameter = 3 m, height = 1.25 m, water level 0.5 m). Both tanks were supplied with filtered flow-through seawater of 34 ppt at a flow rate of 100 L min^{-1} and maintained in a simulated natural light regime. Fish were fed standard commercial pellets at the feeding regime appropriate for size and temperature via an automated feeding system.

After a minimum 3-week acclimation period, fish were PIT tagged using one of two methods: surgical intraperitoneal insertion or injection in the operculum musculature. All fish were part of a separate delousing trial (ethics approval #23818 by the Norwegian Food Authority) for which PIT tags were used for individual recognition. A total of 798 salmon were tagged ranging from 159 to 595 g in weight and 170 to 398 mm fork length (FL). Mean weight ± SEM of the operculum tagged fish was 345 ± 4 g ($N=350$), while mean weight of the intraperitoneally tagged fish was 332 ± 5 g ($N=448$). All fish were tagged with 12.5 mm long × 2.12 mm diameter (tag volume = 44.12 mm^3), weighing 106 mg in air and 104 mg in water, full duplex (FDX) PIT tags designed for subcutaneous or intramuscular implantation in animals (RFID solutions, Norway, www.rfid-solutions.no/). Prior to tagging, fish were lightly sedated (metacaine 10 mg L^{-1}, FinquelVet®, Western Chemical Inc., Washington DC, USA). Individuals were then captured with a bucket and transferred to an anaesthetic tank (100 mg L^{-1} FinquelVet®) where they were held until unresponsive to manual handling.

For intraperitoneal tagging, sedated fish were held ventral side up and a scalpel was used to make a small incision in the abdomen, approximately one fin length anterior to the pelvic fins. The PIT tag was then inserted into the abdominal cavity. For opercular tagging, sedated fish were positioned laterally with the ventral side facing away from the tagger. On the dorsal edge of the operculum musculature, a preloaded needle was inserted at an approximately 20-degree angle into the soft tissue (Fig. 2). After tagging, fish were immediately transferred by bucket back into the holding tank in a common garden design and visually monitored to ensure they all fully regained consciousness, which was determined as upright swimming. Tank 1 contained 200 intraperitoneally tagged fish and 200 operculum tagged fish, while tank 2 contained 248 intraperitoneally tagged fish and 150 operculum tagged fish.

Approximately 4 days after tagging, fish were exposed to one of two treatments, either (i) mechanical delousing [12], or (ii) procedural control. Prior to treatment, light sedation (10 mg L^{-1} FinquelVet®) was added to the holding tank. Fish were then randomly captured via bucket and either moved onto the delousing apparatus and then

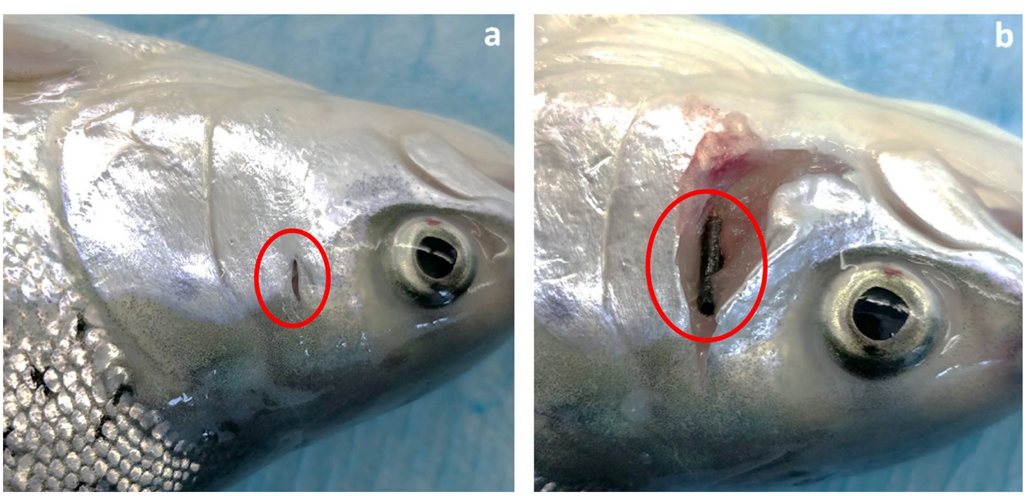

Fig. 2 Position of operculum tags. Image **a** shows the relative point of insertion, while **b** shows the final position of the tag within the muscle tissue. Both are circled in red

to a sedative bath (mechanical delousing treatment) or transferred directly to a sedative bath (procedural control). Delousing consisted of the fish sliding down a 6 m channel on roller bars during which they passed below four rows of jets which expel water at a pressure of 0.6 bar to dislodge lice from the skin. Once sedated, fish were then scanned for PIT tags, lice counted, measured (length (cm) and weight (g)) and scored for welfare using the operational welfare indicators recommended by Noble et al. [8]. Fish were then returned to a holding tank via bucket. Treatment was performed in groups of approximately 100 divided among 4 runs of 25 fish randomly timed in blocks, so each tank was processed in four batches resulting in a total of eight groups. Fourteen days after treatment, all fish were euthanized via immersion in overdose anaesthetic (100 mg L^{-1} FinquelVet®) checked for PIT tags both with a reader and by hand (to ensure tags were not missed by the reader), lice counted and scored for welfare. Throughout the trial, tanks were checked for mortalities and rejected tags daily. In total, excluding fish which lost tags prior to treatment or were injured during the delousing process, there were 305 intraperitoneally tagged fish which were deloused, and 107 procedural controls, while 255 operculum tagged fish underwent delousing and 79 procedural control (Table 2).

Statistics

All data analyses were carried out using R version 3.6.1. (R Core Team 2019). Mortality was calculated by dividing the number of dead fish by the total number of fish initially tagged. Fish which lost their tags were excluded from the mortality analysis. Tag loss was calculated by dividing the number of fish which retained their tags by the initial number of tagged fish. Mortalities were excluded from the tag loss calculation. Condition factor (K) was calculated as: K = body weight (g)/for k length^3 (cm) × 100 [11]. Standard procedures for data exploration were followed to ensure that there were no outlying observations and to test for collinearity among explanatory variables [14]. As a result, fish weighing less than 200 g were excluded from analyses as there were few individuals.

To determine which potential explanatory variables influenced tag loss, mortality and welfare, generalized linear mixed models (GLMMs) were fitted using the lme4 package [2] with *fish weight* (g), *condition factor* (K), *PIT location* (intraperitoneal or operculum) and *treatment* (deloused or control) as explanatory variables. As these data consist of multiple observations of fish from the same tanks, mixed effects models were used with *group (1–8)* as a random intercept factor. Tag loss and mortality were modelled using a binomial distribution, while for welfare scores which are continuous and positive a gamma probability distribution was assumed. Model assumptions for each model were evaluated by plotting Pearson residuals versus the fitted values and versus each covariate [15].

Table 2 Sample size for each PIT tagging method and treatment group

Tank	Group	Treatment	*N*	
			Peritoneum	Opercula
1	A	Deloused	49	41
1	B	Deloused	50	51
1	C	Deloused	41	54
1	D	Control	48	43
1	x	Tag lost pre-treatment	12	11
2	A	Deloused	54	36
2	B	Deloused	59	35
2	C	Deloused	52	38
2	D	Control	59	36
2	x	Tag lost pre-treatment	24	5

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Authors' contributions
TO planned the experiment, collected and analysed data and wrote the manuscript; GM wrote the manuscript; MS planned the experiment and collected data; FO planned the experiment and commented on the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
This study was approved by the Norwegian Food Authority (Ethics approval #23818).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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