Animal Biotelemetry

TELEMETRY CASE REPORT

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Assessment of PIT tag retention, growth and post-tagging survival in juvenile lumpfish, *Cyclopterus lumpus*

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Abstract

Background: Passive integrated transponder (PIT) tags are used to study the movement and behaviour in populations of a wide variety of fish species and for a number of different applications from fisheries to aquaculture. Before embarking on long-term studies, it is important to collect information on both short- and medium-term survival and tag retention for the species in question. In this study, 90 juvenile lumpfish (10–20 g, 30 fish per replicate tank) were implanted with 12.5-mm FDX PIT tags.

Results: Tag retention, growth rates and survival were compared to those of fish subjected to handling only (90 fish, 30 per replicate tank). Overall survival was 100% during the 28-day monitoring period, and tag retention was 99%.

Conclusions: Results indicate that retention rates of 12.5-mm PIT tags in juvenile lumpfish are high, and there is no significant effect on growth rates or survival in a hatchery environment.

Keywords: PIT tag evaluation, Lumpfish, Lumpsucker, Aquaculture, Growth, Survival

Background

Passive integrated transponder (PIT) tags are a low-cost method for marking individuals for breeding applications and mark-recapture studies as well as offering a non-obtrusive method to observe progress, behaviour and movements of tagged individuals using antennae. PIT tags have been used on many different species of fish since the 1980s e.g. [1–6]. Earlier studies were primarily concerned with tag retention and effectiveness of the technology with a variety of body locations tested as insertion points (e.g. peritoneal cavity, opercular muscle and dorsal muscle) [2]. As the use of PIT tags grew more widespread, focus turned to the potential adverse effects of PIT tag implantation in fish [7, 8]. In addition, evaluation of the compatibility of PIT tag use in different species was considered. A common side-effect of PIT tagging is the encapsulation or rejection of tags over the long term [9], with one study resulting in the migration of the tag from the intraperitoneal cavity into the body cavity. This led the authors to conclude that intramuscular insertion was preferential [10]. In contrast, some studies have suggested that intramuscular insertion can lead to greater tag rejection (e.g. [11]), while others found no effect on survival, relative daily growth or tag retention between experimental groups (control, peritoneal cavity and dorsal musculature, [12]).

A suite of parameters has been used to assess the effects of tagging fish, including survival, growth, condition and cortisol level. Growth and condition are the least subjective parameters to measure for assessing tagging effects. Depressed growth has been reported following surgical implantation of PIT tags into the intraperitoneal cavity in several species (e.g. [13, 14]). An evaluation of survival, growth and condition in juvenile Atlantic salmon *Salmo salar* (L.) tagged with two tag sizes (23 mm vs 32 mm) found reduced growth and some tag rejection

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of the larger size [15]. Similarly, Smircich and Kelly [16] noted slower growth due to 'heavy' tags (tag 9.3% of body weight) initially, but compensatory growth occurred as the trial progressed. Skov et al. [5] studied mortality, condition, specific growth rates and tag expulsion and found no difference between test groups of roach *Rutilus rutilus* (Linnaeus, 1758) of average weights between 20.6 and 24.7 g using 23-mm PIT tags inserted into the body cavity. Lower et al. [17] found increased levels of environmental cortisol levels in holding tanks post-tagging, which reverted to normal levels of 12-h post-tagging.

Understanding the effects of tagging on more subjective parameters (e.g. swimming ability, behaviour) may provide a more thorough picture when examined in addition to the parameters discussed above. However, species-specific reaction to tagging should be considered. For example, one study found that maximum burst swimming speeds were significantly lower in PIT-tagged fish compared to the control group [18]. In contrast, an experiment with rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) noted no significant effect on swimming performance between experimental groups [19]. In addition, no significant differences between control and PIT-tagged groups for either the latency to resume feeding or the amount of food eaten have been noted in several species [19].

Lumpfish *Cyclopterus lumpus* (L.) are highly effective at removing sea lice *Lepeophtheirus salmonis* (Krøyer, 1837) from farmed Atlantic salmon [20–22] and are being deployed in sea pens in large numbers [23–25]. Special feeds, refuges/shelters and husbandry techniques are required to maintain condition and facilitate effective sea lice removal. The ability to tag, observe and monitor individual fish provides a valuable insight into fish behaviour. For example, LeClerq et al. [26] used a passive-acoustic telemetry system to track individual cleaner fish in salmon pens. By tracking and visualising fish movements, the authors highlighted the critical role of refuges/shelters in cleaner fish husbandry and welfare. Future studies will benefit from the ability to track individual animals over time using PIT tags, and they will have the additional benefits of accounting for individual variation and the reduction in the number of test subjects required.

Before undertaking research using PIT tags, it is imperative that pilot studies be conducted to determine any potential behavioural or physiological consequences to the organism due to tag insertion. It is also necessary to ensure that the data generated are representative for untagged conspecifics and that the tagging itself does not impede or impair health and welfare of the fish [27]. Feasibility studies on tag acceptance are strongly encouraged when no detailed data are available on the species of interest, both for ethical considerations and validation of results [28].

Although many lumpfish studies state that individuals have been tagged, there are no empirical peer-reviewed data, to the authors' knowledge, on the effects of tagging on lumpfish growth and survival. Therefore, for ethical consideration and validation of results prior to the initiation of a large-scale tagging study, it was deemed necessary to undertake an evaluation of this species acceptance of PIT tags. This study was developed to consider the suitability of 10–20 g lumpfish for intraperitoneal tagging with 12.5 mm × 2.1-mm PIT tag by assessing: (1) survival of tagged fish up to 1-month post-tagging (2) growth and condition of tagged fish and (3) tag retention for juvenile lumpfish.

Materials and methods

Lumpfish origins and rearing

Lumpfish eggs were sourced from an Icelandic hatchery and transported to Ireland. These were disinfected (Pyceze®, as directed by the manufacturer) on arrival at Carna Research Station (CRS) (National University of Ireland, Galway) and maintained in standard lumpfish egg incubation cones (recirculating system). On hatching, larvae were reared in 440 L square glass re-enforced plastic (GRP) tanks, in the same recirculating system as the egg cones, at 10.0 ± 0.6 °C. After 2 months, all fish were transferred to flow-through tanks (1200 L) for ongrowing. Feed (Otohime®) was administered using belt feeders (recirculating system) and Linn® automatic feeders (flow-through system). Feeders were set to distribute feed for approximately 14 h per day, while belt feeders released feed on a continuous basis; the Linn® feeders were set to dispense feed at regular intervals (every 15–20 min). A simulated photoperiod of 16 h light and 8 h dark was maintained using overhead lights on a timer. Fish were fed during light hours at various rates (1–10% of total biomass) depending on fish size. All tanks were cleaned regularly to prevent build-up of waste, and water quality data (temperature and oxygen) were taken twice daily using a hand-held Oxyguard® Probe. Water in the recirculating system was exchanged at a rate of 20–25% every other day to maintain water quality, a standard practice developed for this system in CRS.

Experimental tanks and set up

Lumpfish were housed in either rectangular tanks ca. 200 L or square tanks (1200 L). All tanks were fed with ambient flow-through sea water that had been filtered via both drum and UV filtration systems. Ambient water temperature ranged between 2.9 and 8.8 °C throughout the duration of the study. Fish were fed for the duration of the

tagging study using Linn® automatic feeders as described above, with a photoperiod of 16:8 light/dark.

At 175 days of post-hatching, 180 fish were randomly assigned to a treatment group and to one of six tanks (1200 L). Treatment groups were (a) anaesthetised and measured (handled treatment) or (b) anaesthetised, measured and PIT tagged (PIT-tagged treatment). A decision was made to maintain three groups of tagged and three groups of non-tagged/handled fish separately (i.e. 61,200 L tanks) because it was unknown whether tags would be lost or not. Mixing both tagged and untagged fish in a tank would prevent researchers from determining tag retention, should any tags be lost. This approach was adapted from several studies [5, 10, 29]. Each tank contained 30 fish, with three replicate tanks giving a total of 90 fish per treatment. All fish were starved for at least 24 h prior to the start of the experiment (standard practice in CRS for finfish sampling to reduce stress) and feeding was initiated 24-h post-tagging [30].

The automatic feeder for one of the control tanks tripped, resulting in the tank being fed partially in the dark during the first week of the study. Only one tank of control fish was affected, and this was corrected in subsequent weeks.

Tagging method

As the lumpfish is a weak swimmer with a short body, preference was given to intraperitoneal tag insertion rather than dorsal muscle insertion so as not to further impede swimming ability. Additionally, as the body cavity was large enough to accommodate a 12.5-mm tag (ca. 0.1 g), it was preferable to the opercular muscle which was considered too small for 10–20 g fish. Juvenile lumpfish (10–20 g) were anaesthetised by immersion in a 100 mg/L solution of Tricaine® [31]. Tag mass was between 1 and 2% of fish mass. When the fish became non-responsive (approximately 50 s), they were removed from the anaesthetic, measured and weighed to the nearest 0.1 mm (total length) and 0.1 g (total weight). At this point, fish from the handled treatment were placed in an observation tank until 30 fish had been measured. Once all 30 fish had recovered, they were placed in their respective study tank. This was repeated for each replicate tank.

Fish in the PIT-tagged treatment were tagged with a 12.5×2.1-mm full duplex (FDX) PIT tag adapting methods developed by Biomark® (see Fig. 1a). In brief, each fish was held ventral side up with the tail pointing away from the operator. A preloaded, sterile needle was inserted (bevel down) posterior to the edge of the suction disc to the side of the mid-ventral line (Fig. 1a). This ensures that the tag is inserted away from the heart and other vital organs. The angle of the needle was approximately 10°–20° from the axis of the fish body. The depth of needle penetration was dependent on the size of the fish, with larger fish requiring a deeper insertion as their skin was thicker. Failure to pierce the skin fully results in a tag failing to insert fully (D'Arcy and Bolton-Warberg, personal observation). Each fish was placed in an observation tank similar to the handled treatment. Once 30 fish had been tagged and subsequently recovered, they were placed into a study tank. This was repeated until three PIT-tagged treatment tanks were filled.

Short-term observation

A short-term observation (15 min) of behaviour in both handled and PIT-tagged fish was undertaken by monitoring recovery from anaesthesia for each individual immediately following the anaesthetising/tagging in the recovery tank. A subjective baseline for 'normal' behaviour was determined by an operator with 10+ year's

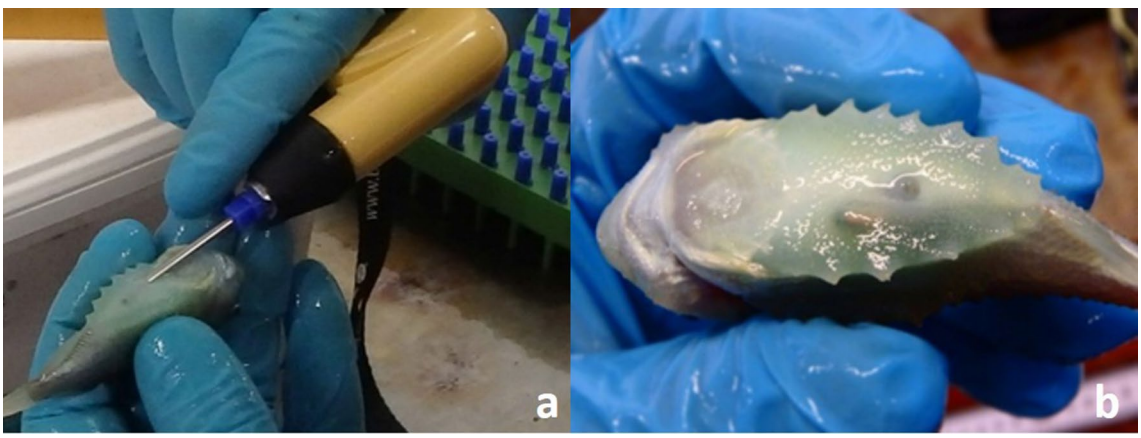

Fig. 1 a and b. Intraperitoneal tagging of juvenile cultured lumpfish (10–20 g) showing **a** position of fish during tagging, insertion point, and angle of needle and **b** final position of tag in a fish tagged at a very shallow depth

husbandry experience of marine finfish (5 years with lumpfish). For this assessment, 'normal' behaviour was defined as behaving in a manner identical to pre-tagging/anaesthetising, i.e. recovery of an upright body position, an ability to stick to flat surfaces, and swimming ability in short bursts typical of lumpfish.

Medium-term assessment

Fish were reared for 28-day post-tagging (time frame also reported in e.g. [5, 16, 30]) in the study tanks with ambient seawater. Any mortalities were removed and recorded daily. During post-tagging on days 8, 14, 22 and 28, all fish were removed from their tanks, and measured for total length and weight without anaesthetic as is standard practice for lumpfish. All fish in the PIT-tagged groups were scanned for tags using a PIT Biomark® 601 hand-held reader, and wounds visually inspected externally. Insertion points with healed skin, healed muscle and the internal muscle appearing closed were deemed sufficiently healed to prevent tag loss, while wounds that remained open were still considered vulnerable to tag loss (e.g. [32]). Any bruising, blood or missing tags were noted, and a subsample was photographed.

Data analysis

All data analysis was carried out using Minitab® 17, with a significance value (a) of 0.05, unless otherwise stated.

Survival and tag retention

Survival (%) was calculated as:

$$S(\%) = 100 \times (\text{final number of fish})/(\text{initial number of fish}).$$

A Chi-square test was used to compare survival among treatment groups.

Tag retention (%) was calculated for each tagged group as:

$$\text{TR}(\%) = 100 \times \left(\text{number of fish that retained their tags}\right)/\left(\text{number of fish tagged}\right)$$

The proportion of fish in each tagged tank with wounds that were sufficiently healed, and assumed likely to retain their tag, was calculated. A score was adapted from Thorstad et al. [33] and is described as follows: wound at time of tag insertion as muscle is visible and open = 0% healed; deeper layers of skin and muscle sealed but outer layers of skin still scarred/open = 50% healed; no perceptible wound = 100% healed.

Growth and condition

Nested analysis of variance (ANOVA) was used to test for potential differences in weight of fish between tagged and handled fish, where replicates were nested within treatments (e.g. as used in [34]) on the final day of the experiment. In addition, in order to evaluate differences between experimental groups over the duration of the experiment, a pairwise comparisons t test was undertaken on mean weights and specific growth rate (SGR).

Specific growth rate of each group (tank) was calculated according to the formula of Houde and Schekter [35]:

$$\text{SGR} = 100 \times \left(e^{g} - 1\right)$$

where $g = (\ln(W_2) - \ln(W_1))/(t_2 - t_1)$ and W_2 and W_1 are mean weights on days t_2 and t_1, respectively.

Condition factor (K) of individual lumpfish (calculated at each weighing interval) is defined as:

$$K = 100 \times \left(W/L^3\right)$$

Final condition factors and SGR for all treatment groups were compared using t tests.

Results

General observations

The short-term assessment of fish post-anaesthesia and tagging revealed that complete recovery (i.e. returning to a state of behaviour [swimming, suction and orientation] identical to that which was observed prior to anaesthetic and tag insertion by an experienced lumpfish producer/researcher) in both handled and PIT-tagged fish occurred within 2 min. During the medium-term assessment, needle insertion wounds were found to be at least 50% healed within one week and 90–100% healed after 2 weeks in most fish (Fig. 2). One tagged lumpfish had a tag coming out of the wound from day 22 but had not been lost by day 28. Two tagged fish exhibited small bulges at their wound, but the skin around the wound was healed.

Survival and tag retention

Survival of all lumpfish (both tagged and control groups) for all six tanks was 100% at 28 days of post-tagging.

Tag retention was 100% at 28-day post-tagging for all PIT-tagged individuals. All fish were checked for wound health on day 28 and found to vary from small bulges near the wound to fully healed skin (100% healed). Overall, 99% of fish had wounds that were classified as 'skin 100% healed' and no longer at a risk of tag loss. The remaining lumpfish (one individual) had the tag protruding and was considered to still be at risk of tag loss.

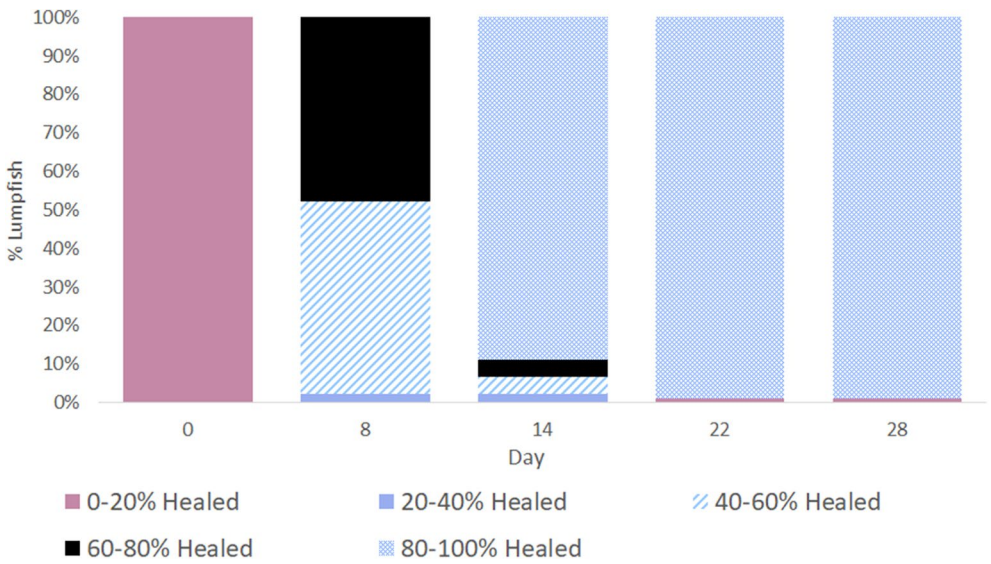

Fig. 2 Bar chart showing the percentage of lumpfish with wounds ranging from fully healed (day 14–28) to newly incised (day 0)

Growth and condition

Initial weights for the experimental population ranged from 9.7 to 21.0 g, with an average of 13.9 ± 2.9 g. There was no significant difference in total weight between replicates (nested ANOVA, $p > 0.05$) or treatments (nested ANOVA, $p > 0.05$) at the beginning of the experiment (Fig. 3). Similarly, there was no significant difference between the final weights of the two treatments or replicates within treatments (nested ANOVA, $p > 0.05$). Pairwise comparison tests between handled and tagged fish (all replicate tanks combined) revealed no significant difference in mean body mass or specific growth rate for the duration of the experiment (t test, $p > 0.05$). In all tank populations, fish had the lowest growth rates in the period between day 8 and day 14 (Fig. 4) which was attributable to lower than normal temperatures experienced during this time (see Additional file 1).

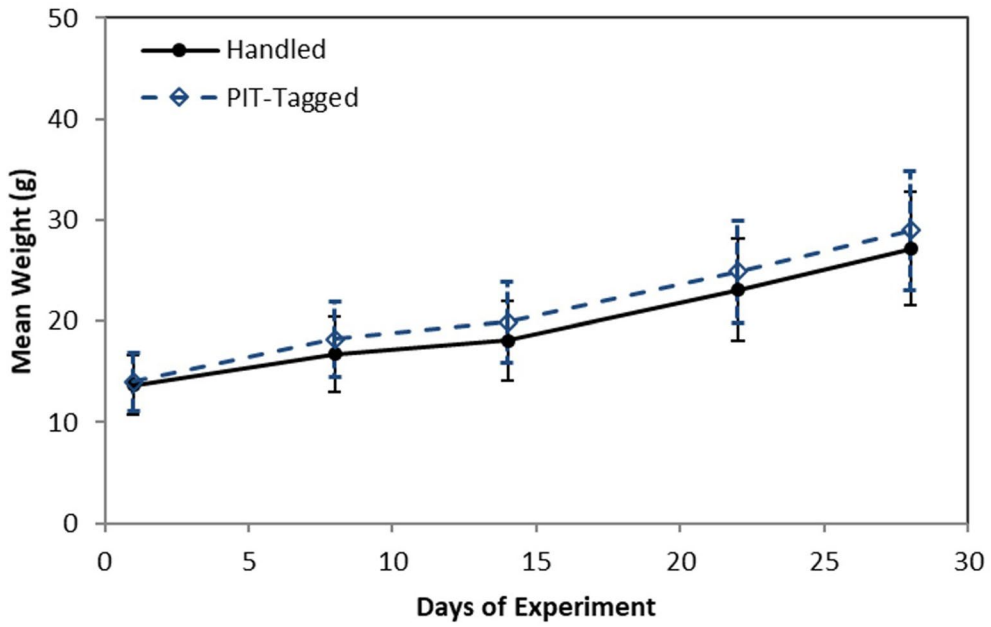

Fig. 3 Mean weight ± SD (g) of juvenile lumpfish in handled and PIT-tagged groups. Means calculated from 90 fish (30 per replicate tank)

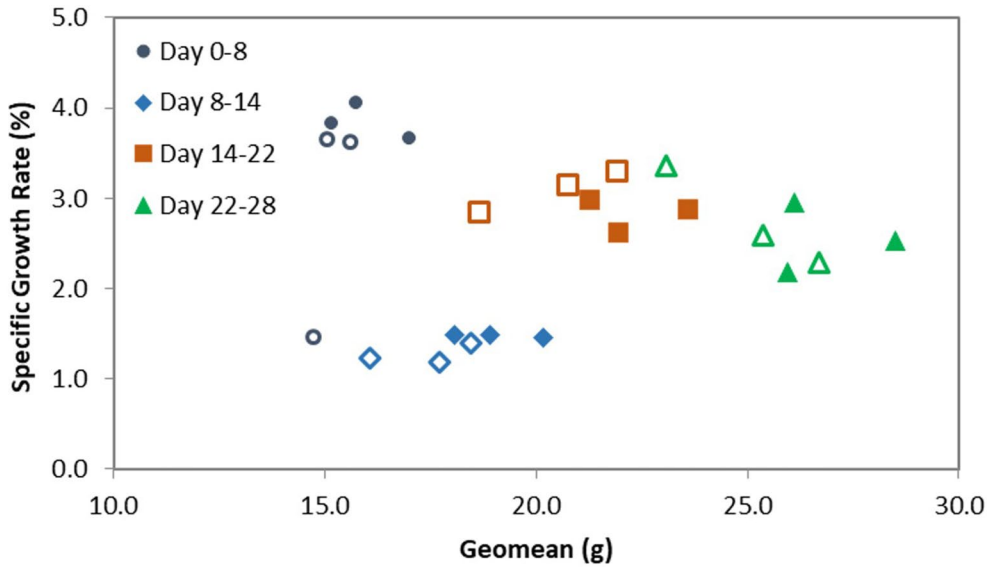

Fig. 4 Relationship between specific growth rate (%) and geomean (g) of juvenile lumpfish in handled and PIT-tagged groups. Each data point represents the data for one tank of fish (6 points/tanks per sampling period). Each colour/shape combination represents a different time period, unfilled shapes indicate control groups, and filled shapes indicate tagged groups (four different sampling periods)

Final condition factor varied between 0.8 and 1.3, with an overall mean of 1.0 ± 0.1. Over the duration of the experiment, there was no significant difference in condition between handled and tagged groups (all replicate tank data combined, t test, $p > 0.05$).

Discussion

This study evaluated the suitability of intraperitoneal implantation of 12.5 mm × 2.1-mm PIT tags on small lumpfish (ca. 10–20 g). The objectives are deemed to have been met based on the results, namely survival, tag retention and growth which were comparable between tagged and control groups. Additionally, wounds healed well and recovery from anaesthesia occurred without any ill effects in all experimental groups. To facilitate comparisons between studies Additional file 2: Table S1 includes results from research using similar parameters to the present study to assess the suitability of PIT tags.

There are several considerations prior to the insertion of PIT tags in a finfish species for the first time. In the past, and in the absence of data, the weight of the tag relative to the weight of the fish (tag/bodymass) has generally been recommended to be no more than 2%. However, Jepsen et al. [28] concluded that the maximum useable tag size is driven by the specific study objectives, the tagging method and the species/life stage involved, although there is not a generally applicable rule relating to the tag/bodymass relationship. They also noted that the impact on behavioural effects, as well as the role of the environment and fish condition at the time of tagging, should be considered.

There is clear evidence that species-specific differences exist with tagging method, tag size and fish size all acting as contributing factors. In some species (e.g. [19, 32, 36]), a minimal effect on survival and health has been observed in tagged individuals. In others (e.g. [13, 37]), tagging resulted in high mortality. The recovery from anaesthesia of the test subjects in this study was assessed using their general behaviour including swimming ability. Their ability to swim was not used to assess the effects of inserting a 12.5-mm PIT tag, however, because lumpfish are relatively poor swimmers that typically spend most of their time adhering to a surface. Studies on other fish species have used swimming ability as an effective evaluation of tag effects on movement, for example [18, 19].

Various methodologies for measuring wound healing in tagging studies have been utilised. In the present study, wound healing was described as percentage healed, which was an easy method to employ. The time it took for the insertion wounds to heal for the lumpfish in this study was comparable to, or faster than, other fish species [7, 10, 11, 37]. However, it should be noted that in one study [37], smaller fish took significantly longer to heal than larger fish, and overall survival was poor (60%) despite the relatively quick healing times. A comparison of implantation methods found significantly reduced wound healing for fish tagged by incision compared to syringe [38]. Wound healing is not measured in all

tagging studies [5, 13, 14, 30, 32, 36, 39–42] (Additional file 2) and may not always be indicative of ill effects of tagging. However, wound healing should be monitored in the interest of fish welfare and ensured that the wound has no significant impact on survival, tag retention, growth, condition and general health.

Overall, there was 100% survival of fish in this study throughout the study period comparable to other studies [5, 7, 10, 28, 30, 40]. The intraperitoneal tagging method employed in this study was adapted from best practice (i.e. bevel down, acute angle, to the side of the central line and away from the anterior); therefore, the probability of tag-induced mortality was reduced. In some longer studies, survival was marginally lower; >95% in Fuller and McEntire [41] and 92% in Simard et al. [32] at 41–118-day post-tagging. In one study, both fish and tag sizes had a clear impact on mortality, with mortality only occurring in smaller individuals (fork length < 103 mm) tagged with the larger tags (32 mm vs 23 mm) [15]. These results highlight the importance of undertaking an evaluation of tag suitability prior to undertaking a large-scale experiment.

Tag retention was very high among the fish in this study, with similar results found in several other studies [5, 7, 32, 41]. For example, a study of juvenile Atlantic salmon (80 to 135-mm fork length) by Larsen et al. [15] found that retention rates of 23-mm PIT tags with and without suture closure were 100% and 97%, respectively, while retention of larger 32-mm PIT tags without suture closure was 69% primarily due to the large tag-to-body size ratio. Another study found a tag retention rate of >80% [30], which was not quite as high as this study. Retention rates can be influenced by several factors, for example, the angle of insertion [9], tag size [15] and fish size [37] as cited in Grieve [42]. The high retention rates observed in this study are likely related to the thin needle used for tag insertion, the relatively small tag size (12.5 mm) and the low tag burden (1–2%).

The marginally reduced growth exhibited by one of the control groups during the first week is explained by a non-synchronised automatic feeder. The mean weight for this group was smaller than all the other experimental groups from this point, yet it had similar growth rates once the feeder had its settings corrected. Specific growth rates were reduced in all six groups during the second week of the trial, which coincided with the lowest water temperatures experienced throughout. No significant differences in growth and condition were observed between the tagged and control groups in this study. This compares with other tagging evaluations in which no negative impact was found regardless of tagging location, e.g. [7, 10, 14, 30, 41], while another assessment demonstrated reduced growth rates over the first 3 days of post-tagging but normal growth thereafter [13]. In contrast, two separate studies have found that growth of small fish was negatively affected by tagging [14, 37]. It is probable that tag burden effects were avoided in the present study by selecting larger-sized fish. Other studies have found that larger PIT tags can affect growth via tag burden [14, 27, 43]. Tag burden occurs when the tag significantly adds to the fish's mass. It is noted when the growth of fish is hindered due to an inability to move efficiently and added energy requirements to compensate for the tag's mass [44]. Tag burden in the present study was between 1 and 2%. Other trials have examined the effects of tag burden on fish behaviour and physiology and have concluded that many species can cope with tag-to-body weight ratios of up to 5% without being negatively affected [45–48]. If larger tags were required with juvenile lumpfish or similar sized tags were to be used on much smaller lumpfish, an additional evaluation study would be required.

Conclusion

When all aspects of this trial are considered, namely a relatively quick healing time, very high survival and tag retention, and no negative effect of growth and condition, it can be concluded that small lumpfish are suitable candidates for tagging with 12.5-mm PIT tags. These results are attributable to adherence to best practice for intraperitoneal tagging, low tag burden, small tag and needle size and candidate species. Prior to all manner of future studies in lumpfish condition/health, welfare, feeding behaviour and broodstock selection, the researcher, having followed the methods described herein, will be reassured that all efforts to tag and track an individual fish using 12.5-mm PIT tags will have minimal adverse effects on the physiology and behaviour of the lumpfish. The ultimate goal of the emerging lumpfish aquaculture industry is to produce juveniles that adapt well to deployment in salmon pens and are efficient at delousing farmed salmon while maintaining the health and welfare of both salmon and cleaner fish [49].

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40317-019-0190-6.

Additional file 1. Water temperature (°C) throughout the study period for lumpfish either handled or PIT tagged, and dotted lines indicate sampling dates. Note drop in temperature between days 8 and 14.

Additional file 2.

Abbreviations

PIT: Passive integrated transponder; FDX: Full duplex; GRP: Glass re-enforced plastic; ANOVA: Analysis of variance; SGR: Specific growth rate; Tukey's HSD test: Honestly significant difference test; BIM: Bord Iascaigh Mhara; KGS:

Knowledge gateway scheme; EMFF: The European Maritime and Fisheries Fund.

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Authors' contributions
JD, SK, TM, JH and MBW made substantial contributions in data acquisition. JD, MBW and DJ made substantial contributions to conception and design. MBW undertook the analysis and interpretation of data. MBW and DJ sourced funding for this study. JD and MBW produced initial drafts and all authors contributed to edited versions of the manuscript. All authors read and approved the final manuscript.

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

Ethics approval and consent to participate
The welfare of the subjects was foremost in considerations from the design of this trial through anaesthesia and tag insertion. As such, the number of subjects chosen was minimal while maintaining statistical robustness. The severity of tag insertion under anaesthesia was deemed to cause low levels of discomfort. The Health Products Regulatory Authority (HPRA, the National Competent Authority) is responsible for following the provisions of the relevant legislation in the EU (Directive 2010/63/EU) and in the Republic of Ireland (SI No. 543 of 2012) on the protection of animals used for scientific purposes. Both the Marine Institute and The National University of Ireland Galway have Breeder/User/Supplier authorisations under HPRA of Ireland, and as such have licence to breed, use and/or supply animals for scientific or educational purposes. Additionally, all operators have completed training in fish tagging, are compliant with best practice, and hold individual authorisations from HPRA.

Consent for publication
All authors have given final approval of the version to be published.

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

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