

Size dependent effects of passive integrated transponders (PIT-tags) on early life stages of salmon: a preliminary literature review

Knut Wiik Vollset, Robert J Lennox, Shad Mahlum, Joacim Näslund, Martin H Larsen, Eva B Thorstad, Danielle Frechette, Bengt Finstad, Jan Grimsrud Davidsen



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Forfattere: Knut Wiik Vollset, Robert J Lennox, Shad Mahlum, Joacim Näslund, Martin H Larsen, Eva B Thorstad, Danielle Frechette, Bengt Finstad, Jan Grimsrud Davidsen

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Sammendrag: Passive Integrated transponders, eller PIT merker, er en utbredt merkemeter for å individ merke Atlantisk laks og sjørret. Merkene brukes blant annet til å studere sjøoverlevelsen og veksten til laks i havet i en rekke studier. Et viktig element i slike studier er å forstå hvor stor merkeeffekten er og hvor små fisk som er mulig å merke med gitte merkestørrelser. Lenge- eller vekt-til-merkestørrelse ratio er ofte bruk til å definere minimums størrelse for merking. Samtidig er det velkjent at mange andre faktorer kan være like avgjørende for gjennomføre en vellykket merkestudie; for eksempel behandling, slipp sted og merkemeter. Samtidig er minimums størrelse viktig fordi det er en objektiv måte å definere en dyrevelferdsmessig grense for merking av fisk.

I oktober 2018 ble det holdt en workshop koordinert av NORCE og Havforskningsinstituttet med forskere fra Norge og Danmark ved havforskningsinstituttet i Bergen. Målet med workshopen var å dele erfaringer og data som kan bli brukt til å definere en minimums størrelse for merking av Atlantisk laks med PIT merking. I denne preliminnære rapporten oppsummerer vi funnene fra denne workshopen og et enkelt litteratursøk.

For å komme til en enighet om et minimumsmål ble deltagerne på workshopen bedt om å definere et minstemål for merking av Atlantisk laks (i gaffellengde) i en anonym online spørreundersøkelse, etter å ha lest oppsummeringen fra litteratur gjennomgangen. Ingen av deltagerne definerte en føre var grense på merkestørrelse over 130 mm for 23 mm PIT merker og 85 mm for 12 mm. Over 16 av 18 deltagerne definerte minstemålet som under eller lik henholdsvis 120 og 80 mm for 23 og 12 mm PIT merker. Det ble dermed kommet til en konsensus om at 120mm gaffelengde for 23 mm PIT merke og 80 mm gaffelengde for 12 mm PIT merke bør være preliminnære føre var minstegrenser for merking med PIT merker ved bruk til å studere marin overlevelse og vekst hos Atlantisk laks. Denne rapporten er en preliminnær rapport til en pågående detaljer systematisk gjennomgang og meta-analyse av litteratur knyttet til merke effekter av PIT merking.

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Size dependent effects of passive integrated transponders (PIT-tags) on early life stages of salmon: a preliminary literature review

Authors

Vollset KW¹, Lennox RJ¹, Mahlum S¹, Näslund J², Larsen MH³, Thorstad EB⁴, Danielle Frechette⁴, Finstad B⁴, Davidsen, J. G.⁵

¹NORCE, LFI - freshwater biology, Nygårdspporten 112, 5006 Bergen, Norway

²Stockholm University, Department of Zoology, Svante Arrhenius väg 18 B, S-106 91 Stockholm, Sweden

³Danish Centre for Wild Salmon, Brusgårdsvej 15, 8960 Randers SØ, Denmark

⁴Norwegian Institute for Nature Research (NINA), Postboks 5685 Torgarden, 7485 Trondheim, Norway

⁵NTNU University Museum, 7491 Trondheim, Norway

SUMMARY

Passive integrated transponder (PIT) tags are commonly used to individually mark salmonids for assessing marine and freshwater survival, habitat use, and other aspects of life history essential for persistence of the species. Effects of PIT-tags on is pivotal to understanding any bias that would compromise the validity of a study. Usually a minimum threshold in length (or weight) is used to define the size of tag that would be appropriate for the species and life stage being studied. Tag-to-fish weight/length ratio is a standard way of defining the potential impact of tags on the fish, although other factors can be equally important, such as handling and post-operative care, wound closure method (e.g., suture, surgical glue), and the placement of the tag (e.g., internal vs. external). Notwithstanding, the size of the tag relative to the individual is an important metric that should be used to prevent tagging fish with tags that are too large. Further, animal welfare committees around the world often use this simple metric to ensure that the animal welfare is being upheld. Consequently, resolving debate around allowable minimum size thresholds for marking salmon with PIT-tags is important for fish welfare and the future of salmon research programs.

A workshop discussing this issue was held in Bergen Norway in October 2018. The goal of the workshop was to share experience and published and unpublished data that can be used to draw up recommendations on a lower threshold for tagging of Atlantic salmon based on the impacts of using PIT-tags in small salmon. In this preliminary report, we summarize the findings from the workshop. A preliminary literature review was conducted based on a database search and identification of studies from the participants at the workshop.

After summarizing the results from relevant identified studies, the participants of the workshop was asked to answer an anonymous questionnaire on the precautionary lower size limit (in fork length) for tagging Atlantic salmon smolt with 12 and 23 mm PIT-tags. None of the participants suggested a higher precautionary lower size limit than 85 mm fork length for 12 mm PIT-tags and 130 mm fork length for 23 mm PIT-tags. A consensus of 80 mm fork length for 12 mm PIT-tags and 120 mm fork length for 23 mm PIT-tags was reached as a precautionary limit for tagging of Atlantic salmon smolt. This report is the preliminary report for a more detailed systematic review and a meta-analysis.

INTRODUCTION AND BACKGROUND

Tagging of animals always has negative impacts; even when tagging a large animal with a small tag, this will negatively impact some fish. The ethical questions of whether it is justifiable to tag usually depends on how negative the impacts are, weighted against the need and value of the study and results.

Fish have long been important subjects of tagging studies because they are inherently difficult to observe and count. Tagging fish with unique marks including PIT-tags permits individual recognition and has permitted insight into the habitat preferences, migratory routes, and demographics of species that have broad ranges. Atlantic salmon were one of the earliest species used in tagging studies to reveal their spatial distribution and habitat use. Salmon are among the most culturally and economically important species in the world and their range overlaps with many areas of significant human impact in Europe and North America. Owing to pollution, habitat alteration, and other impacts, Atlantic salmon have declined throughout their range and their conservation in the wild is a priority for many researchers and management agencies. Electronic tagging plays a critical role in tracking salmon in the wild and feeding back important data to stakeholders and managers with which to make informed decisions.

Atlantic salmon migrations to the sea are risky and although many smolts may exit the river, only a small percentage returns. There are many factors that contribute to the likelihood of a smolt returning to their natal river as mature adults and understanding these factors can contribute to improved resource management. Calculations of sea survival have been made possible by tagging programs that implant migrating smolts with tags so that they can be detected upon return and the sea survival of a cohort may be estimated. Such data makes direct contributions to establishing fishing regulations, instigating management measures, and developing long-term time series with which to investigate temporal trends in abundance and study long-term viability of salmon populations.

Passive integrated transponder (PIT) tags are commonly used method to individually mark salmonids (Atlantic salmon, brown trout and Arctic charr) for assessing marine and freshwater survival, habitat use, and other aspects of life history essential for persistence of the species. The main advantages of PIT-tags compared to traditional mechanical tags are

- PIT tags can be registered automatically using antennas.
- PIT tags have much smaller negative impacts on the tagged fish than external tags like Carlin tags.
- Compared to other electronic tags, PIT-tags have a lifetime exceeding that of the fish, whereas acoustic and radio transmitters suited for small fish like salmon smolts only last for a few months (i.e. not long enough to record sea survival for salmon, because they stay at sea for one or more years before they return to rivers).
- PIT tags have small size and low weight compared with other commonly used electronic tags.
- Developed tagging methods allow researchers to tag hundreds of fish within a working day.
- PIT-tags are also much cheaper than for instance acoustic transmitters (~14-25 NOK per PIT tag, versus about ~2000-5000 NOK per acoustic transmitter), hence budgets often limit the number of tagged fish with acoustic transmitters to only a few individuals per study.



Photo 1 Pictures of migrating salmon smolt. Photo: Tore Wiers

The main drawbacks associated with use of PIT-tags for automatic registration are that

- the detection range is limited
- the detection distance from an antenna may vary depending on the electric field created by the antenna and the ambient electrical noise in the vicinity (e.g., power lines), the type of PIT-tags used (i.e., full vs half-duplex) and the size of the PIT tag.

For many ecological studies involving fish, it is necessary to maximize the likelihood of detecting individuals with PIT-tags while limiting any potential negative impacts that would produce unwanted biases. As a result, the most commonly used sizes of PIT-tags are 12 and 23 mm (length).

The goal of tagging fish in the wild with PIT-tags is usually to understand the survival, and in some cases behavior and growth of the fish. In laboratory studies, PIT-tags are often used to obtain individual growth trajectories and survival. The effect of the PIT-tags on these parameters is pivotal to understanding any bias that would compromise the validity of a study. Usually a minimum threshold in length (or weight) is used to define the size of tag that would be appropriate for the species and life stage being studied. Tag-to-fish weight/length ratio is a standard way of defining the potential impact of tags on the fish, although other factors can be equally important, such as handling during and post-operative care, wound closure method (e.g., suture, surgical glue), and the placement of the tag (e.g., internal vs. external). Different PIT-tagging methods are also used – using scalpel and inserting the PIT tag or use of pre-loaded tags in sharp syringes deployed directly into the fish (See photo 1 and 2).

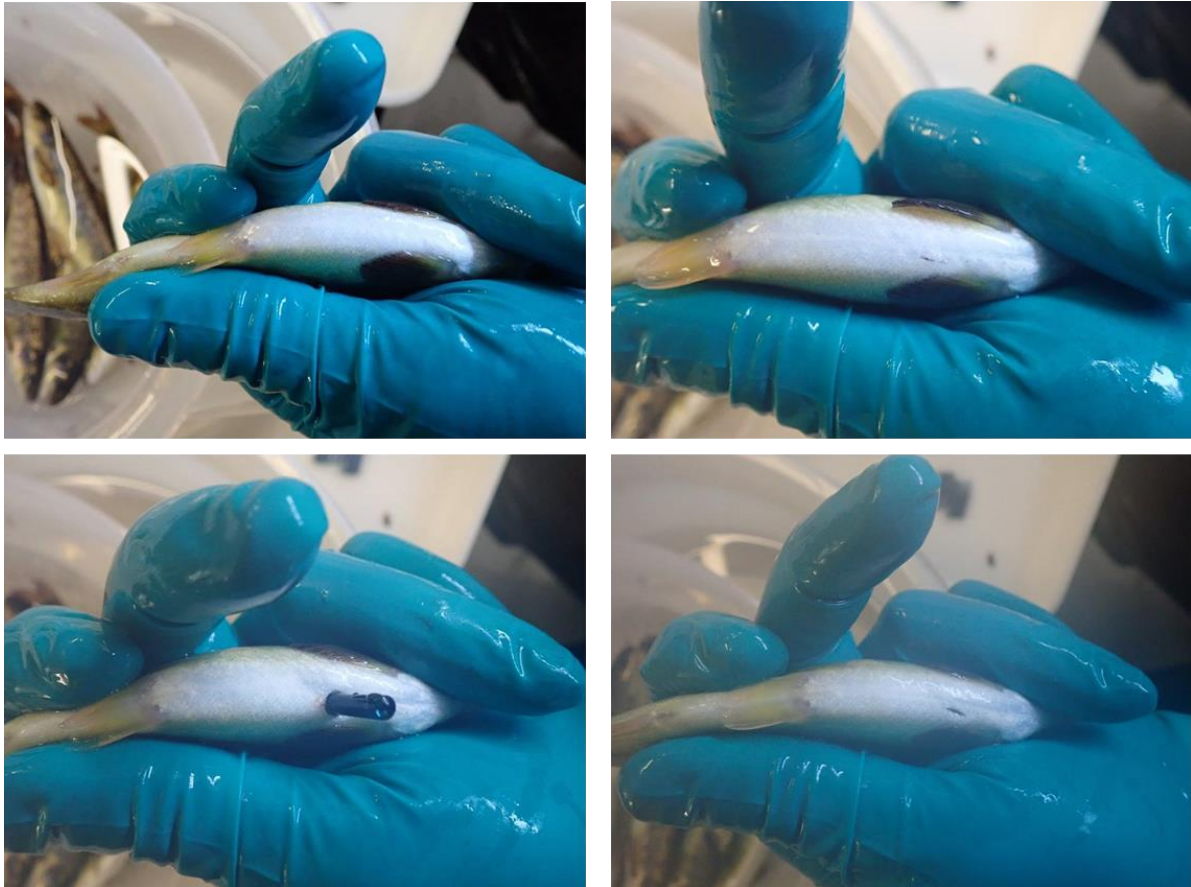


Photo 2 Pictures of tagging of Atlantic salmon smolt with 23 mm PIT-tags using scalpel without suture. Photo: Tore Wiers



Photo 3 PIT-tagging of Atlantic salmon smolts with pre-loaded PIT-tags (www.biomark.com).

Notwithstanding, the size of the tag relative to the individual is an important metric that should be used to prevent tagging fish with tags that are too large. Further, animal welfare committees around the world often use this simple metric to ensure that the animal welfare is being upheld. Consequently, resolving debate around allowable minimum size thresholds for marking salmon with PIT-tags is important for fish welfare and the future of salmon research programs.

On the other hand, the effect of setting a lower threshold unnecessarily high, can also have animal welfare implications. For example, if the size limits are set so that it is not possible to use a certain sized tag, research programs will have to switch to smaller and less effective tags. Consequently, to be able to design the same studies with equivalent power, more animals

will have to be tagged. This goes against the principle of *reduction* in animal welfare ethics. An example of setting the lower size limits too low can be seen by comparing the given lower size limit for use of 23 mm tags in Norway (140 mm) with the size distribution of wild salmon in a typical salmon river on the west coast of Norway (Vosso - Figure 1). This illustrates that in this river almost 80% of the fish would not be eligible for tagging using this lower size limit, in practice rendering the tagging study not feasible.

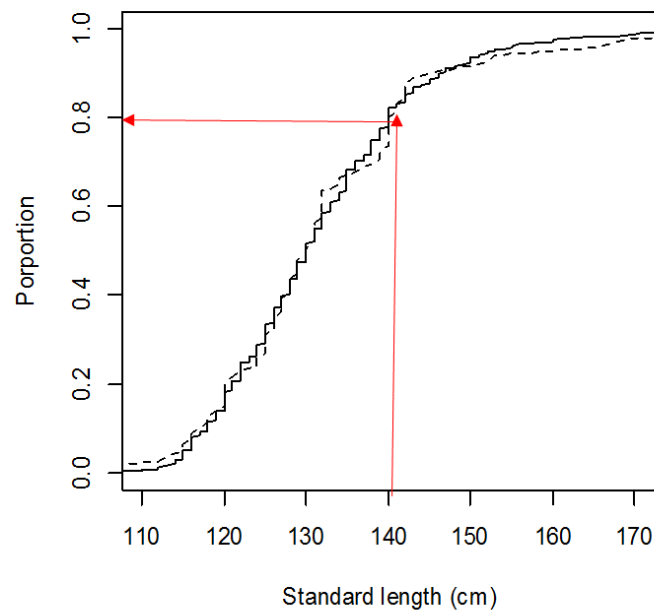


Figure 1 Cumulative size plot of fish tagged (solid line) and detected (dashed line) at a floating antennae in the river Vosso (modified from Barlaup et al. 2018). Red arrows indicate given lower size limit for tagging with 23 mm tags in Norway. Note that there is no sign of size selection between the released and detected fish in this graph.

Determining the lack of an effect in a study is usually more difficult than obtaining support for the presence of an effect. In statistical terms, that is, being sure that failure to reject the null-hypothesis is not because you do not have enough data to provide evidence for the direction of the effect, rather than there being no effect of treatment. This is often termed “statistical power”, and is often ignored in studies when the null-hypothesis does not lead to any action. In the case of setting a lower threshold for tagging, “no-effect” does lead to an action – it means that the researcher will tag a fish down to a certain size. Generally, single experiments have relatively low power (depending of course on the number of replicates and the study design). A way of increasing power to detect a general effect is to do a meta-analysis where multiple studies are pooled together and (usually) the inverse of the variance estimates of each study is used to weight the importance of each study. In this way, statistical power increases because the conclusion is based not on one single study, but multiple studies.

The goal of this project is to apply a systematic review and a meta-analysis to study the effect of PIT-tags on the growth, survival, and behavior of salmon smolts. The study was initiated by inviting experts that have worked with PIT-tags on Atlantic salmon, sea trout and Arctic charr to a workshop hosted by Norwegian Research Centre (NORCE) and Institute of Marine Research (IMR) in Bergen. The goal of the workshop was to share experience and published and unpublished data that can be used to draw up recommendations on a lower threshold for tagging of salmon based on the impacts of using PIT-tags in small salmon. In this preliminary report, we summarize the findings from the workshop. We acknowledge that this is not a complete systematic review and we will continue the work towards a full review that can be

submitted to an international peer-reviewed scientific journal for publication. However, the reason for publishing this report prior to the completion of the review is to provide additional information on preliminary findings as basis in evaluation of new applications. Therefore, this report is intended to be used to guide animal welfare committees in their work when giving allowances to scientific studies using PIT-tags on young life stages of salmon based on present knowledge. Furthermore, the report will be used as a basis for the initiation of systematic review and a meta-analysis.



Photo 3 Pictures of migrating salmon smolt. Photo: Tore Wiers

METHODS

An invitation letter to a workshop was submitted to individuals identified through a preliminary search of the literature and communication with other researchers working with PIT-tagging studies in Norway. The invitation list was potentially biased by this preliminary search and it was therefore specified in the letter that invitees should distribute the letter to other contacts that might have interest in the workshop. The original letter is given at the end of this report. The Workshop was held at the Institute of Marine Research in Bergen on 30 October 2018 (Photo below).



Photo 1 Workshop participants. Photo: Siv Kristin Eikås Hovland, IMR

During the workshop it was decided that the outcome of the meeting should focus on two objectives: (1) a preliminary report summarizing status quo based on the key literature used to identify the appropriate size limit for tagging salmon with PIT-tags, and (2) a more comprehensive meta-analysis based on a systematic review. This report is the resulting preliminary report. To address this a simple search on AFSA was conducted with the following search terms linked with both OR and AND statements

- A) smolt* OR hatchery OR laksesmolt OR salmon OR *Salmo salar* OR post-smolt*
- B) PIT OR half-duplex OR full-duplex OR passive-integrated-transponder* OR passive integrated transponder*
- C) Effects OR laboratory OR swimming endurance OR survival OR growth

This search provided a list of 353 publications, from which duplicates were removed (using the title). All publications that described experimental studies (either field or laboratory studies, identified based on their title) were downloaded. Abstracts were read to confirm that these were experimental studies that focused on tagging effects such as retention, growth, survival or behavior. Studies using only coded-wire tags (CWT) were removed. A list of studies was then created and circulated among the participants. Participants were asked to add potential studies not identified in the preliminary search. A full list of relevant publications was then made. Following this, a core group of the participants (KWV, SM, RJL, JN, ML) read the publications and summarized the main findings from the studies. Finally, after circulating the text, a formal consensus was discussed and agreed upon among all the participants in the

group related to precautionary size limit for use of PIT-tags to tag Atlantic salmon. To reach this consensus, each participant was asked to define a «precautionary lower limit» for 12 and 23 mm based on their expert opinion and after reading the summary of studies. This was done using an anonymous online application.

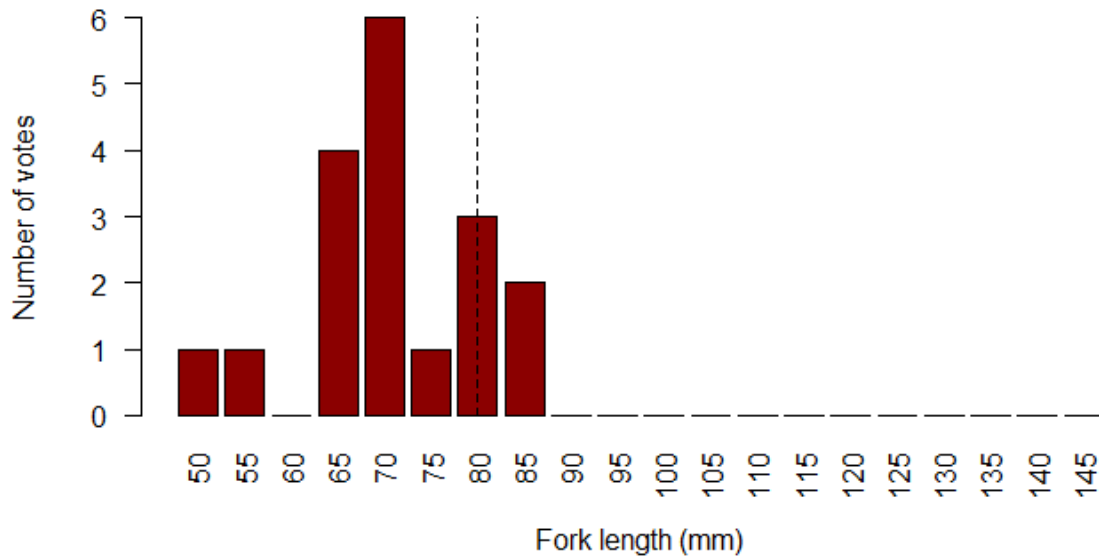
SUMMARY OF STUDIES IDENTIFIED BY EXPERT GROUP

A summary of the findings related to tag effects from each of the publication is found in appendix I. Not all, but some of the studies make concrete recommendation for lower size limits when tagging young salmonids. Peterson et al (1994) assessed the growth and survival of overwintering juvenile coho salmon using 11 mm PIT-tags, and recommended the use of 11mm tags in individuals 65mm (FL) and greater. Larsen et al. (2013) conducted a 35-day laboratory study and concluded that intracoelomic implantation of 23 mm PIT-tags without suture closure of the incision provides a useful method for individual marking of Atlantic salmon larger than 99 mm FL. Conversely, the authors recommend that 32 mm PIT-tags should not be used for marking Atlantic salmon 80-135 mm FL due to high mortality, high tag loss rate and reduced growth. Gries and Letcher (2002) evaluated tag retention and survival of age-0 Atlantic salmon in the laboratory following surgical implantation with 12 mm PIT-tags, and more generally concludes that surgical implantation of 12 mm PIT-tags is a viable technique for marking juvenile Atlantic salmon. Acolas et al. (2007) assessed survival, growth and tag retention following PIT-tagging of juvenile brown trout in a 27-day laboratory experiment, and conclude that brown trout \geq 57 mm FL can be tagged by injecting 11.5 mm PIT-tags into the peritoneal cavity with negligible effects on survival and growth, though the tag loss rate was quite high (20%). Ostrand et al. (2011) conducted a four-month laboratory experiment with various salmonids and PIT tag sizes. Their main conclusion was that there were only minor, if any, effects on long-term survival, growth and physiology in salmonids with a fork length of $>$ 120 mm and $>$ 20 g, using 12 to 23 mm tags. Tiffan et al. (2015) investigated effects of 8-, 9-, and 12 mm PIT-tags on growth, survival and tag-retention of chinook salmon juveniles in three size classes. The main conclusion from their study was that biologically relevant effects on growth and survival were negligible using i) tags up to 9 mm for 40-49 mm fish, and ii) tags up to 12 mm for 50-69 mm fish, over the first month post-tagging.

CONSENSUS

After reading the literature summary all participants was asked to define a “precautionary” lower limit in fork length (mm) for tagging of Atlantic salmon smolt with 12 and 23 mm PIT-tags. The results are given in the figure below. For 12 mm the opinion varied from 50 to 85 mm with a center around 65 mm. For 23 mm the opinion varied from 100 to 130 mm, with 8 out of 13 suggesting a limit of 100 mm.

Precautionary lower limit for tagging salmon with 12 mm



Precautionary lower limit for tagging salmon with 23 mm

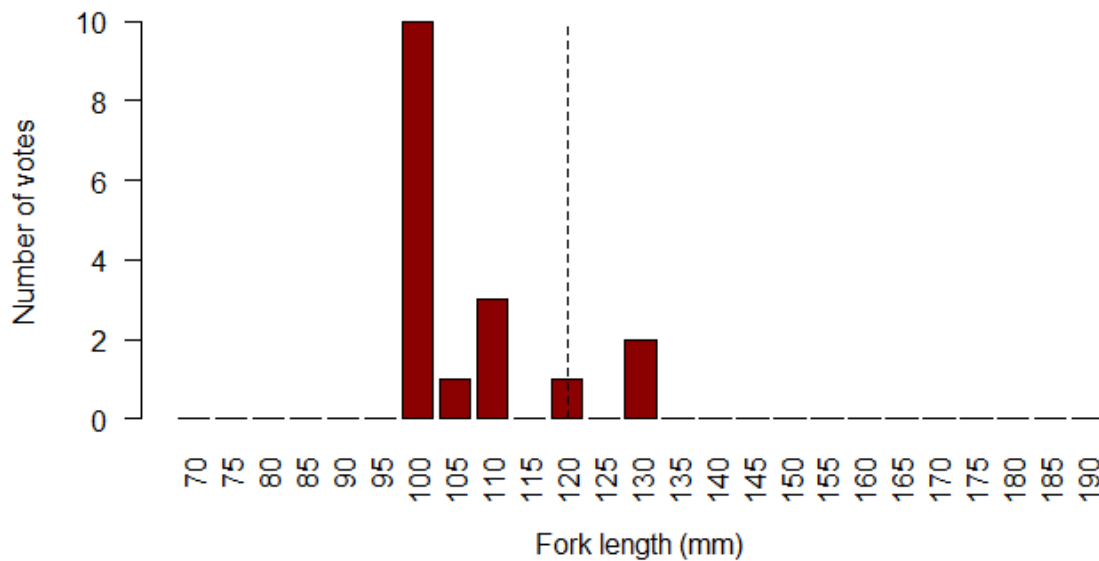


Figure 1 Results from questionnaire from participants on workshop or on mailing list, based on the question “Minimum size limit 12 mm PIT tag (fork length)?» (upper panel) and «Minimum size limit 23 mm PIT tag (fork length)» (lower panel). Dashed line indicate consensus among participants.

CONCLUSIONS

It is important to note that this is not an exhaustive literature review of all studies related to effects of PIT-tagging on salmonid, but a preliminary study based on an expert group mainly from Norway. The group has identified numerous key studies related to tagging effects of PIT-tags on young salmonids. The report will be used as a foundation for a systematic review and meta-analysis on the topic. Based on the available data at hand the group suggest a preliminary “precautionary” lower limit for tagging Atlantic salmon smolt with 12 mm PIT-tags to be 80 mm and lower limit of tagging salmon with 23 mm to be 120 mm, when using PIT

tags for marine survival studies based on the objective criterion that 16 of 18 of the participants suggested this or a lower threshold levels. Participants was not asked to differentiate for a lower limit in studies using PIT tags in for example growth or laboratory studies (which in theory could be lower), and these limits should not be viewed as an all-encompassing limit, but a preliminary limit before the results from the meta-analysis is finished.

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APPENDIX – SUMMARY OF STUDIES

Riley et al (2018) caught Atlantic salmon in rotary screw trap and doubled tagged fish with CWT and PIT-tags. The manuscript failed to report the size of PIT-tag used, but former studies published by the same authors used 12 mm tags. However, the present study only tested the effect of fish handling and tagging with CW compared with PIT-tags. The effect varied with winter temperature and the time of day that fish were tagged. There was a marginal non-significant effect of length on likelihood of recapture. However, the effect of fish length on the tagging effect was not significant.

Steig et al. (2003) compared downstream migration of Pacific salmonids, (Chinook salmon, sockeye salmon, and steelhead), tagged with small or large acoustic transmitters or PIT-tags. PIT-tags measured 11.5 mm and were placed in fish measuring on average 141 mm, 193 mm, or 115 mm for Chinook, steelhead, and sockeye respectively. Steelhead, the largest fish on average, had the highest survival (29%) whereas the smallest, sockeye, had only 3.95% survival. The test, however, was not a controlled experiment to estimate the effects of the PIT-tags on fish survival or swimming and there was no control group. The comparison was with small (17 mm) and large (20 mm) acoustic transmitters. There was evidence that chinook salmon tagged earlier in the season had lower survival when tagged with PIT-tags than with the larger acoustic transmitters, but all other comparisons were not significant. Steelhead tagged early in the season traveled faster downstream between two reaches when tagged with PIT-tags than with large acoustic transmitters but not small transmitters, nor was there a difference between the transit times recorded between groups of acoustically tagged fish. Late in the season, however, the opposite occurred with steelhead traveling faster with PIT-tags than with small transmitters. The study concludes that some differences can manifest in survival and behaviour of tagged fish, but there was equivalent evidence that PIT-tags were no? better or worse than the larger and heavier acoustic tags, suggesting that the tag size was not an important parameter.

Knudsen et al. (2009) compared survival of PIT-tagged Chinook salmon with fish identified using non-specific marks (i.e. adipose fin clip). The salmon were cultivated in a hatchery in the Yakima River, Washington State, many of which are tagged to investigate survival. The Chinook salmon smolts used in the study had an average length of at least 100 mm (fork length) and the PIT-tags were 12 mm in length. Upon return as adults, PIT tagged fish were the same length as non-tagged fish except for four-year-old adults, which averaged 1.1 cm shorter in length than untagged counterparts. They report a significant PIT tag-induced mortality with a mean value of 10.3% for all five brood years of the study. However, the study did not address size-related tagging effects

Brown et al. (2010) also investigated the effects of double tagging with PIT-tags and acoustic transmitters on Chinook salmon smolts. Smolts were divided into control and treatment groups with three size bins, 80-89, 90-99, and 100-109 cm FL and held for 30 d in a laboratory. A significant difference was recorded for survival and growth of fish 80-89 cm FL but not for the larger size classes at the end of the study. Tag expulsion rates were also greater among smaller PIT tagged fish. According to FishBase, 1 cm FL = 1.034 cm TL for chinook salmon, so the smallest group was approximately 82-92 cm TL, followed by 93-102 cm and 103-113 cm TL. Because fish were double tagged, results are somewhat confounded when interpreting the results; tag burdens were 11.5, 8.2, and 5.7% of the bodyweight. Size of the PIT tag was not reported but the weight was 0.10 g in air.

Brakensiek and Hankin (2007) applied mark-recapture survival methods to investigate the effects of tagging coho salmon. Coho salmon juveniles as small as 55 mm FL (no FishBase conversion available) with 11.5 mm PIT-tags. Winter survival was higher for juvenile coho salmon PIT tagged in October than November and in both months, there was an effect of fish length as determined by Cormack-Jolly-Seber modelling. Small fish of ~55 mm FL survived at a rate of 12% compared to 100% for fish 83 mm FL and larger. However, it is not clear to what extent small fish naturally have lesser survival and without a control group it is difficult to ascertain whether this is a tag effect given that it could equally be an effect of poor condition yielding overwinter mortality.

Peterson et al (1994) assessed the growth and survival of wild overwintering juvenile coho salmon using 11 mm PIT-tags (though not directly indicated in the methods) to tag individuals (mean ~ 75mm; min 53mm) and compare to individuals tagged with sequential CWT. No significant differences in growth or survival was detected at the smallest size range (53 – 70 mm). The authors went on to indicate that they were able to recover a 58 mm PIT-tagged smolt, but because there were so few fish at these smaller size ranges they could not preclude the potential effects of PIT-tagging and *therefore recommended the use of 11mm tags in individuals 65mm (FL) and greater.*

Dare (2003) assessed the long-term (3 – 4 weeks) survival and tag retention (including causal factors) of 145,000 PIT-tagged (12 mm tags) hatchery reared spring Chinook salmon. Tagged population length metrics were not mentioned but seem to be from <100mm to 170mm with 84% of the population less than 130mm. Overall reported mortality was less than 1%. There did not seem to be size-related mortality and most of the mortality was attributed to the hatchery environment due to the delay between tagging and mortality. However, he could not determine the extent that stress related to tagging contributed to mortality. Tag retention was also very high (> 99%) and the expulsion of tags was attributed to the experience level of personnel, as 85% of the shed tags came from a single tagging station where 4 of 6 people had no previous experience tagging fish.

Hockersmith et al (2003) compared the migration rates and survival between radio-tagged and PIT-tagged yearling Chinook salmon. The study is more focused on the potential effects of radio-tags on Chinook salmon (length range 127 – 285 mm FL) and used PIT-tags as a reference. Three groups were included in the study, which differed by tag type and tagging method: 1) gastric implants with a dummy radio transmitter (18mm long X 7.3mm diameter) encapsulating a PIT tag, 2) surgical implantation of sham radio transmitter (18mm long X 7.3mm diameter?) in the body cavity, and 3) surgical implantation of a PIT tag (assumed to be 12mm based on the 12 gauge hypodermic needle used). For both treatment groups with dummy radio tags, survival was significantly lower compared to PIT-tagged individuals that had migration rates exceeding 22.5 km/day but similar survival among groups where migration rates were less than ~17km/day.

Sigourney et al (2005) assessed the growth and survival of PIT-tagged (11.5 mm) wild stock Atlantic salmon parr (FL 60 – 69 mm) reared in a hatchery. They found significant evidence of

decreased survival during the first 2 months (82% in tagged vs 96% of control where 62% of mortalities occurred within the first 5 days). Furthermore, survival was positively correlated with length, with small fish having a decreased probability of survival. No significant difference in growth between treatments and controls though the authors noted a slight depression in growth.

Larsen et al. (2013) conducted a 35-day laboratory experiment to investigate the potential effects of 23 and 32 mm PIT-tags (both tag sizes measured 3.85 mm in diameter) on three different size classes of juvenile Atlantic salmon: I: 80-99 mm, II: 100-119 mm, III: 120-135 mm FL. The tags were surgically implanted into the peritoneal cavity through a small incision (4-5 mm long) made with a scalpel. A group of salmon tagged with 23 mm PIT-tags also had their tagging incision closed with absorbable suture. During the experiment, all salmon tagged with 23 mm PIT-tags survived, while 14% of the fish tagged with 32 mm PIT-tags died. Salmon tagged with 32 mm PIT-tags had significantly lower growth rate in terms of mass compared to the sham (surgery, but no tag implanted) and control (no surgery and no tag implanted) groups across all size classes. Non-sutured salmon between 80-99 mm tagged with 23 mm PIT-tags had lower growth rate compared to untagged (control and sham) and sutured individuals tagged with 23 mm tags. However, no differences in growth were found between untagged and 23 mm PIT-tagged salmon larger than 99 mm (i.e., size classes II and III). Tag retention rate was 97% for 23 mm PIT-tags when the tagging incision were left to heal without closure and no 23 mm tags were lost when the incisions were closed with suture. The tag loss rate for 32 mm PIT-tags was 31%. The tagging incisions without suture closure were generally well healed, while 35% of the sutured incisions were inflamed and/or infected with fungus. Hence, the authors advise against the use of sutures to close the tagging incisions. Based on these results, *it is concluded that intracoelomic implantation of 23 mm PIT-tags without suture closure of the incision provides a useful method for individual marking of Atlantic salmon larger than 99 mm FL*. Conversely, the authors recommend that 32 mm PIT-tags should not be used for marking Atlantic salmon 80-135 mm FL due to high mortality, high tag loss rate and reduced growth.

Foldvik and Kvingedal (2018) investigated the long-term (533 days) retention rate of 12.5 mm PIT-tags in hatchery-reared Atlantic salmon. The salmon were tagged at the pre-smolt stage (late juvenile freshwater stage) and the experiment was terminated after the fish had spent 1 year in seawater. The average length at tagging was 163 mm FL. The PIT-tags were inserted into the peritoneal cavity using a Biomark gun implanter with pre-loaded needles. Overall, the tag retention rate was 91% at the end of the study. Potential effects of PIT-tagging on growth and survival were not evaluated. In addition, fish that died during the experiment were not scanned for PIT-tags.

Gries and Letcher (2002) evaluated tag retention and survival of age-0 Atlantic salmon (46-182 mm FL; average: 115 mm FL) in a laboratory experiment following surgical implantation with 12 mm PIT-tags (measuring 2 mm in diameter). The tags were implanted into the peritoneal cavity through a small incision (approximately 2 mm) made with a scalpel. At nine months post-tagging, tag retention rate was 99.8% and survival was 94.3%. No control or sham-operated group was included in this study and growth was not evaluated as an endpoint. Nevertheless, *the study concludes that surgical implantation of 12 mm PIT-tags is a viable technique for marking juvenile Atlantic salmon*.

Acolas et al. (2007) assessed survival, growth and tag retention following PIT-tagging of juvenile brown trout in a 27-day laboratory experiment. The PIT-tags used were 11.5 mm long and 2.1 mm in diameter. The hatchery-reared trout measured 41-70 mm FL at tagging with an average length of 48.8 mm FL. PIT-tags were injected into the peritoneal cavity using a 12-gauge hypodermic needle. At the end of study, mortality was higher for PIT-tagged trout compared to untagged fish. Mortality for the PIT-tagged fish was related to length at tagging, with larger fish having the highest probability of survival. Larger brown trout also showed the

lowest tag loss rate. Using logistic regressions, the authors found that the probability of survival reached 95% for fish ≥ 52 mm FL at tagging, while tag retention was 70%. For fish ≥ 57 mm FL at tagging survival was above 99% and tag retention was 80%. Growth of the PIT-tagged trout did not differ significantly from that of the untagged fish at the end of the study. *The authors conclude that brown trout ≥ 57 mm FL can be tagged by injecting 11.5 mm PIT-tags into the peritoneal cavity with negligible effects on survival and growth, but the tag loss rate was quite high (20%).*

Ombredane et al. (1998) evaluated the effects of 11 mm PIT-tags (2.2 in diameter) on survival and growth of juvenile brown trout (55-127 mm FL) in a field study. The PIT-tags were injected into the body cavity with a 12-gauge hypodermic needle. To evaluate the effects of PIT-tagging in the field the authors combined this marking method with fin clipping of the left pelvic fin. The trout were captured by electrofishing and released in their captured sector after tagging. Survival, growth and tag retention were determined by resampling the river system. Seven months post-tagging, the tag loss rate was estimated at 3.4%. In addition, PIT-tagging had no significant effects on growth and survival of the trout. *The study concludes that 11 mm PIT-tagging in juvenile brown trout provides a feasible marking technique for ecological studies of trout in their natural environments.*

Ostrand et al. (2011) conducted a four-month laboratory experiment, in an aquaculture-like setting, where they investigated effects of PIT-tags on growth, survival and tag-retention in four species of salmonid parr. Tag sizes used were 12-, 19-, and 23 mm tags in coho salmon (average FL: 131-135 mm) and steelhead trout (average FL: 123-125 mm), 12- and 23 mm tags in bull trout (average FL: 136-141 mm), and 23 mm tags in cutthroat trout (average FL: 149-150 mm). Tags were inserted by hand into the body cavity of anaesthetized fish, after cutting a small opening between the pectoral and the pelvic fin. No significant effects were found in final size four months post-tagging. However, there was a tendency for fish tagged with 23-mm, and possibly 19-mm, tags to grow slightly slower on average during a period of one to two months post-tagging, but overall effects on growth were marginal in a hatchery environment. Mortality did not differ significantly, as compared to controls, for any species. Overall mortality was low in tagged fish ($\leq 3\%$), except for bull trout (tagged fish: 10-15%; controls: 12%). Tag retention was high and did not differ significantly depending on tag size in any species. However, the highest tag loss (11%) was observed in steelhead tagged with 23-mm tags, which were also the group with the smallest body size. For all other groups, tag-loss was lower than 3%. In addition, physiological indicators of osmoregulation, used for smoltification assessment, were investigated in coho salmon, but did not differ significantly between tagged and control fish. *The main conclusion was that there were only minor, if any, effects on long-term survival, growth and physiology in salmonids with a fork length of > 120 mm and > 20 g, using 12 to 23 mm tags. Notably, the authors caution about extrapolating the effects to smolts. They argue that tagging conducted during critical life-stages such as overwintering or migration, could possibly affect results.*

Tiffan et al. (2015) investigated effects of 8-, 9-, and 12 mm PIT-tags on growth, survival and tag-retention of chinook salmon juveniles in three size classes (FL: 40-49 mm, 50-59 mm, and 60-69 mm; 12 mm tags not used for the smallest size-group). The experiment was run over one month in the laboratory, in an aquaculture-like setting. Tags were inserted with syringes in anaesthetised fish; 12-gauge needles for 9- and 12 mm tags, and 14-gauge needles for 8 mm tags. Growth rate in fork-length was reduced due to tagging over the first 7 days in smaller sized fish, but the effects were judged to be marginal from a biological perspective. Overall, no major effects on growth rate were observed during the whole course of the experiment, and all treatment groups were similar in size to controls at the end (day 28). Survival was generally high, ranging from 97.8 to 100%, without indications of relevant body-size or tag-size effects. Tag retention was high (93-99.3%), without apparent effects of tag-size. The highest expulsion rate was found for 12-mm tags in the 50-59 mm size-group, but the 40-49 mm size-group tagged with 9-mm tags had a higher initial tag-burden and higher retention rate than the former.

The majority of expelled tags were lost during the first 7 days. *The main conclusion was that biologically relevant effects on growth and survival were negligible using i) tags up to 9 mm for 40-49 mm fish, and ii) tags up to 12 mm for 50-69 mm fish, over the first month post-tagging.*

Richard et al. (2013) conducted a 60-day laboratory experiment in an aquaculture-like setting to evaluate tagging effects on growth, survival, and tag-retention in age-0+ brown trout parr (two size classes: 50-55 mm FL and 56-63 mm FL), using 12 mm PIT-tags. The tags were inserted either by using a scalpel to make a small incision into the body cavity and thereafter inserting the tag using a needle as a guide, or directly by an injector applied with a needle and a plunger. In the smaller size-group, the fish had generally lower growth rate than controls over the experiment, with seemingly biologically relevant effects. No such general effects were found in fish >55 mm. In the latter size-group, only a short-term depression in growth rate after tagging was found, and this was later compensated. Fish in the smaller size-group also had lower survival (80.7%) than control fish (91.2%). The majority of the mortality occurred within the first 5 days. Survival rates were similar between tagged and control fish when they were larger than 55 mm. Tag retention was also generally higher in the larger size-group than in the smaller (86.6% vs. 79.2%). The experiment also revealed that the person conducting the tagging can affect fish performance and that the scalpel-method for implantation led to lower fish performance than the injector-method. There were also interactive effects between person and method, i.e. different persons tagging perform differently depending on tagging method.

Appendix II List of participants

Name	Institution
Abdullah Madhun	IMR
Bengt Finstad	NINA
Trond Einar Isaksen	NORCE
Vidar Wennevik	IMR
Knut Wiik Vollset	NORCE
Dag Atle Tuft	Mattilsynet
Rune Nilssen	IMR
Shad Mahlum	NORCE
Ørjan Karlsen	IMR
Joacim Näslund	IMR
Martin H Larsen	Vildlaks DK
Robert Lennox	NORCE
Jan G Davidsen	NTNU
Eva Thorstad	NINA
Kjell Utne	IMR
Tormod Haraldstad	NIVA
Harald Sægrov	Rådgivende Biologer
Per Tommy Fjeldheim	IMR
<i>NOT PRESENT AT WORKSHOP</i>	
Bjart Are Hellen	Rådgivende Biologer
Danielle Frechette	Institut National de la Recherche Scientifique
Anders Foldvik	NINA
Eli Kvingedal	NINA
Torstein Kristensen	Universitetet Nord
Ina Birkeland	NORCE
Bjørn Torgeir Barlaup	NORCE
Øysten Skåla	IMR

Open invitation

Workshop on effects on PIT tagging on Atlantic salmon smolt

We hereby invite scientist to participate in meta-analysis of the impacts of Passive Integrated Transponder (PIT) tagging on Atlantic salmon smolts. The study will be a part of the Salmon at Sea project led by Norwegian Institute for Nature Research (SeaSalar). The project is motivated by the discovery that animal welfare committees around the world are using the same scientific papers, but end up with very different conclusions related to lower size limits for the use of PIT tags. Also, size limits has in some cases changed over time even though no new scientific studies has emerged. This can create issues for time-series and comparative studies as the type of tags of the size distribution of fish tagged will inevitably have to be changed. In addition, tagging effect may bias results, and it is therefore clearly important to understand how to minimize potential tagging effects, particularly size-dependent tagging effects.

The principal investigator of this study has been in contact with various scientists – and the general opinion seems to be that the lower size limits for tagging salmon smolts with 23 mm PIT tags is approximately 10-12 mm. This result seems to be based on personal experience from tagging studies, own non-published material or in often cases a study by Larsen et al. (2013). According to Food Safety Authority in Norway (2018), the lower size limit for tagging salmon with 23 mm PIT tags is 14 cm fork length, and 8.6 cm for 12 mm PIT tags. It seems clear that this topic is ripe for a systematic review and meta-analysis that can lead to a unified advice on the use of PIT tags. Such results will be very relevant for the Salmon at Sea project.

Our ultimate goal of the meeting is that the work should result in a peer-review article describing a meta-analysis on the effect of PIT tagging on survival, growth and (potentially) behavior of Atlantic salmon smolts. One of the subgoals is that such a study is to come to a unified advice that can be used by animal welfare committees around the world to set size dependent limits on the use of PIT tagging of Atlantic salmon.

Please respond to this invitation within September 15 if you are interested in participating.

Best regards

Knut Wiik Vollset (PhD)
Senior scientist
Uni Research