Traditional tagging methods for fish can have issues relating to both animal welfare and economic costs. Biometric data such as iris patterns can be captured via digital cameras allowing non-invasive tagging, and inexpensive and rapid analysis. The purpose of this study was to investigate if the iris of Atlantic Salmon (*Salmo salar*) is a suitable biometric template for long term identification of individuals. Atlantic Salmon were individually tagged in the body cavity using PIT-tags at the juvenile pre-smolt stage and the left eye was photographed 6 times over a 533 day period. Description of changes in iris stability was assessed both qualitatively and using iris-recognition software. Identification of individual Atlantic Salmon using the iris was not successful over the entire period, as the iris pattern changed significantly with time. Over a shorter time period (4 months) with frequent samplings, iris software was able to correctly identify individual fish. The results show that iris identification has potential to replace other methods for Atlantic Salmon over short timeframes.

Individual identification of fish is important for scientific research on both wild and farmed fish. Methods for individual tagging of fish are many, but mostly involve placement of external or internal tags that can be read either directly or by electronic equipment (Thorstad et al., 2013). Both the handling of the fish during the tagging process and the tagging itself can have obvious animal welfare issues and could also affect the fish in regard to what is being studied (e.g. growth, behavior or mortality, Jepsen et al., 2015). Finding non-invasive alternatives to physical tagging could reduce the amount of stress and pain fish have to undergo and improve quality of the science produced via reduced tagging effects. For some research identifying individuals using DNA is possible but requires handling the fish to obtain samples and for a large number of fish, the costs involved can be substantial. Biometric identification using patterns or other structures unique among individuals, may for many species of animals be a cost efficient and non-invasive method for mark-recapture.

Biometric identification in humans is well documented with methods as fingerprints, iris, face and voice recognition systems (Jain et al., 2006). Manual photoidentification of individual animals was pioneered in the 1970 on orcas (*Orcinus orca*) (Bain, 1990) and since been used in a range of taxa.

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With the digital revolution in both image and computer processing, automatic identification using biometric algorithms has been attempted on a range of species such as Delta Smelt (*Hypomesus transpacificus*) (Castillo et al., 2019), Rio Grande Cooter (*Pseudemys gorzugi*) (Suriyamongkol and Mali, 2018), Whale Shark (*Rhincodon typus*) (Holmberg et al., 2009), and several mammal species (reviewed in Kumar and Singh, 2016).

When evaluating traits with regard to suitability for use in biometric authentication, the most important factors are universality, uniqueness, permanence and measurability (Jain et al., 2006). For human use other aspects have to be considered, such as social acceptability of the method and possibilities for the method to be circumvented.

Wild Atlantic Salmon has a high cultural and economic importance in Norway, but these wild stocks are under threat from escaped farmed salmon, identified as being the greatest threat to Norwegian wild salmon populations (Forseth et al., 2017). This has led to repeated demands that farmed salmon should be tagged as to both make it possible to remove escapees from rivers and identify the producer that was responsible for the escape. In 2004 a committee appointed by The Norwegian Directorate of Fisheries defined criteria that should be met for potential tagging/identification methods for aquaculture salmon. These criteria focused on that the methods should, 1) not raise animal welfare concerns, 2) not affect marketing or human consumption, 3) be suitable for pre-smolt size fish (5-10 cm), 4) have easily accessible results from analysis/identification both in terms of effort and time, 5) be suitable for a large number of fish and 6) total cost per fish should be low. Norwegian aquaculture companies have been reluctant to implement physical tagging of fish due to several reasons, including concerns about costs related to tagging and subsequent removal of physical tags before the fish reaches the consumer. Biometric identification has the potential to address all of the criteria for tagging/identification methods defined above, using automated pattern-recognition techniques.

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Atlantic Salmon have two obvious templates for biometric identification, melanophore spot pattern and the iris pattern. Melanophore spot pattern of has been used for individual identification of Brown Trout (*Salmo trutta*) (Bachman, 1984) and the potential for the method has been explored for melanophore spots on the operculum of Atlantic Salmon (Stien et al., 2017). However for Atlantic Salmon this method has issues with permanence of the trait, as both change in size of spots and addition on new spots over time (visible in Fig. 1). Further, many individuals have no or insufficient number of spots for the method to work.

Iris recognition is a mathematical pattern-recognition technique that allows rapid matching with high accuracy, see Daugman, (1993, 2009) for details relating to the method. Suitability of iris as a biometric trait for Atlantic Salmon is clear in terms of universality and measurability, but the uniqueness and permanence of the trait have not been previously reported. The only example we have found were the iris pattern is used for individual identification of fish, is in Goldfish (*Carassius auratus*) (Yoshida et al., 2013). In this case line drawings of eye patterns were overlaid and compared, and not the method widely used and recognized as iris recognition.

Here we investigated the potential for use of iris recognition as a long term biometric method for individual identification of Atlantic Salmon.

MATERIALS AND METHODS

In April 2016, 2986 age 1+ hatchery-reared Atlantic Salmon pre-smolt were tagged with 12.5-mm PIT tags (Biomark HPT12). Tags were inserted to the body cavity using a Biomark gun implanter with pre-loaded needles. Fish were anesthetized using benzocaine (20%) 1.5–2 ml/10 l. The fish were kept in a large indoor tank for 533 days and photographed and scanned for PIT number sequence five times during this period (Table 1). Number and timing of samplings were restricted by the Norwegian Food Safety Authority based on animal welfare considerations. We expected the smoltification period, when juvenile salmon undergo physiological changes necessary to transition from freshwater to saltwater, to be the period with the highest chance for substantial changes in the iris pattern. For

this reason, samplings were conducted at shorter intervals early in the experiment. Photos of the fish left eye were taken using high resolution digital single-lens reflex cameras with settings and lenses changing with both size of the fish and experience gained on speeding up the process. A great deal of time was spent at each sampling to adjust flash angles and input of natural and photographic lighting to avoid glares and reflections in the area of interest. Photos were used rather than an iris scanner for two reasons 1) iris software has not been tailored for use on fish, making iris detection and extraction highly variable, 2) to test the method with the same type of data that could be expected in practical use (photos received from anglers etc.). Length of fish was measured for fork length (FL, Table 1), and fish that had shed their PIT tag were removed from the experiment. During the fourth scanning event, 1146 fish were removed from the experiment to avoid crowding in the tank as the fish grew larger.

Iris pictures were processed using iris software from Neurotechnology (VeriEye 2.10 Standard SDK). Initial analysis on a limited number of individuals found no correct matches using pictures from the 1^{st}, 5^{th} and 6^{th} samplings, whereas correct matches were found from the 2^{nd}- 4^{th}. Visual assessment of changes in iris over the time period (Fig. 1), makes this result consistent with the clearly visible changes that occurred. A subset of fish from the 2^{nd}- 4^{th} samplings (n=14) chosen based on having good picture quality on the 2^{nd} sampling, were selected for further analysis. Pictures of these fish on the 2^{nd}- 4^{th} samplings were registered in to a separate database and there after the software was used to match the same images one by one to the database. Manual pre-processing was applied to these images by adding a white ring around the iris to aid the program to correctly extract the iris. This was necessary due to the algorithm having problems detecting the outer limit of the iris. This is almost certainly a consequence of the algorithm being designed for human use and expecting the iris to be surrounded by the white sclera facilitating iris extraction, which is not the case for salmon. During identifying iris images, the false acceptance rate of the program was set to 100% as not to exclude any matching scores. The image matching score is based on the number of features matched

by the algorithm, this score is not impacted by image size (pixels count). The algorithm was not disclosed by Neurotechnology due to its proprietary nature. Qualitative assessment of iris stability over time was also conducted (Fig. 1).

RESULTS

Successful identification of individual salmon based on iris recognition varied within the experiment, with no success for images taken at the 1st, 5th and 6th samplings. Iris recognition was successful for images from the 2nd – 4th sampling. Substantial changes in iris pattern were visible from the parr stage (1st sampling) to the smolt stage (2nd sampling). This was not unexpected, as the juvenile salmonids undergo large physiological changes during smolitification. During the first summer in saltwater (2nd to 4th sampling) changes in iris were less prominent, with the overall pattern being clearly recognizable. The last samplings were spaced out in time compared to the earlier samplings (6 month apart), likely making changes in iris more prominent. Images from the 5th sampling appear to be more similar to images from the 4th sampling than the 6th sampling. At the 6th sampling approximately half of the fish were sexually mature and had developed breeding (nuptial) coloration. Development of breeding coloration in Atlantic Salmon involves base colour of the fish changing from dark back and silvery sides to a more overall brown colour with black, white and red spots. Development of breeding colours also seem to change iris coloration and pattern. Intrestingly, also pupil shape changed thoughout the experiment in most fish.

The subsample of 41 pictures of 14 fish from the 2nd - 4th sampling (May, June and September, one fish had a poor quality picture from the 3rd sampling which was excluded) that were registered in to a separate database and then matched against each other showed positive results. In this test the other pictures of the same fish from the other samplings consistently had the highest scores (Fig. 2). Some fish had matches with other fish, but the scores of these false matches were always lower than

of the true matches. Only two true match scores (11:2-11:4, 11:4-11:2, score 37) were lower than the highest false match score (6:4-8:4, 39) (Fig. 2).

DISCUSSION

Individual identification of Atlantic Salmon using iris recognition was not a viable method for identifying fish over time period spanning the entire juvenile to adult stage using the methods and software applied in this study. No matches were made from the 1st sampling (parr stage) to any of the other samplings. This is not surprising, given the major physiological transformation the fish undergo during smolitification, transforming the fish from a life in fresh water to saltwater, involving changes in appearance from dark brownish with parr marks to a silvery pelagic form. For the 2nd to the 4th sampling (124 days) where fish developed from smolts to post-smolt/pre-adults, successful matching was achieved. Images of a subset of fish (n=14) from the 2nd to 4th sampling, showed that individual recognition of was successful for all fish in this test. For the 5th and 6th samplings no matches were found with images collected at other dates.

Only two true match scores from the subset from the 2nd to 4th sampling (11:3-11:4, 11:4-11:3, 37) were lower than the highest false match score (6:4-8:4, 39). Ranked match scores per image were always higher for true matches than for false. According to the software manufacturer the default value for accepting a score as a true match is set to 48. Using this value would mean that zero false matches would be accepted and eight of 80 true matches would be rejected in our study. These values of false acceptance and false rejections could suggest that the uniqueness of the iris of Atlantic Salmon is close to what is found in of humans, while permanence is not. For comparison, success rate of PIT-tag identification of the fish over the same period was approximately 98% due to some fish shedding their PIT-tags (Foldvik and Kvingedal, 2018). The percentage correct top ranked matches in the subset from the 2nd to 4th samplings (100%) is high compared to biometric studies on other species of fish 80% in Delta Smelt (Castillo et al., 2019), 50.97% in manta rays (*Manta alfredi* and *Manta birostris*) (Town et al., 2013), 81% in Whale Shark and 85% in Atlantic Salmon (Stien et al.,

2017). These numbers are however not directly comparable as all these studies differ in biometric templates, algorithms, duration and number of individuals included.

The positive matches for the subset from the 2nd to 4th samplings span 124 days, whereas the 5th and 6th samplings were 185 and 360 days after the 4th sampling, respectively. It is likely that the increased duration between sampling affects the chance of identification. Also, approximately half of the fish were sexually mature at the 6th sampling and had begun developing breeding coloration. Differences in iris coloration between return migrating adult salmon caught in sea and river fisheries have also been observed (personal observation A. Foldvik). Pictures taken late June 2016 of the iris of salmon caught in the rivers Tana and Neiden, show that 95% of river caught salmon (n= 63) had no blue or blueish green iris coloration. Whereas pictures taken during the same period of sea caught salmon from the Alta and Trondheim fjords the proportion of salmon without any blue or blueish green iris was only 3% (n=33).

Whereas the human iris absorbs light, many marine organisms have evolved partly reflective irides, which in addition to function as a light barrier also camouflages the eye (Gur et al., 2018). Guanine-based crystals in the iris above the absorbing pigmented layer create a complex optical response of reflection and scattering (Gur et al., 2018) that can make the appearance of the iris change with orientation in regards to both camera and light source. Salmon also seem to have partly reflective irides, making both image acquisition and comparison more difficult than for human irides.

Economic founding and design of this experiment was focused on examining the long term stability of the iris pattern, and the analysis of performance metrics such as uniqueness and permanence of iris over shorter time periods has therefore been limited. For situations where fish can be sampled repeatedly over short time intervals, iris recognition has a potential to replace invasive methods. To estimate the number of individuals that could be successfully identified over different durations, a more in-depth analysis of permanence and uniqueness would have to be performed. Reliability of identification could likely be increased by including both eyes and/or combining iris recognition with

melanophore spot pattern recognition. In addition, fish length could be used as a metric to reduce the number of potential matches, applying known maximal growth (shrinkage) rates between samplings. The increasing number of cameras in many Atlantic Salmon pens for different monitoring purposes, coupled with the continuous improvement of machine vision and artificial intelligence should create ideal possibilities for implementing visual biometric techniques for individual identification of farmed Atlantic Salmon.

Although the iris was unsuitable as a template for long-term biometric identification of Atlantic Salmon, over shorter durations the method has potential to replace invasive tagging methods or supplement other biometric identification methods, minimizing tagging effects and improving animal welfare.

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FIGURE CAPTIONS

Fig. 1. Example of images, original and cropped side by side, of an individual Atlantic Salmon from the 1st to 6th sampling spanning a period of 533 days.

Fig. 2. Matching scores from iris recognition software for 41 iris images from 14 fish from the 2nd to the 4th sampling. The numbering given on the axis is a combination of fish individual (1-14), and which sampling the image was collected. So e.g. 3:2 is fish individual number 3 from the 2nd sampling. Scores of iris images matched against themselves were omitted. True and false matches indicated with bold and italic fonts, respectively. The color of circles indicate which individual the image is from and size is scaled to the matching score.