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# Consistent melanophore spot patterns allow long-term individual recognition of Atlantic salmon *Salmo salar*

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The present study shows that permanent melanophore spot patterns in Atlantic salmon *Salmo salar* make it possible to use images of the operculum to keep track of individual fish over extended periods of their life history. Post-smolt *S. salar* ($n = 246$) were initially photographed at an average mass of 98 g and again 10 months later after rearing in a sea cage, at an average mass of 3088 g. Spots that were present initially remained and were the most overt (largest) 10 months later, while new and less overt spots had developed. Visual recognition of spot size and position showed that fish with at least four initial spots were relatively easy to identify, while identifying fish with less than four spots could be challenging. An automatic image analysis method was developed and shows potential for fast match processing of large numbers of fish. The current findings promote visual recognition of opercular spots as a welfare-friendly alternative to tagging in experiments involving salmonid fishes.



Key words: automatic procedure; individual fish; melanin spots; spot development; visual verification.

## INTRODUCTION

Identification of individuals in groups of fish is often essential in scientific studies, particularly when focusing on biological traits and their variability through time. Techniques for marking individuals include fin clipping, freeze brands, tattoos, external and internal tags (McFarlane *et al.*, 1990; Drenner *et al.*, 2012). Although some studies report no difference in growth or fitness due to tagging (Otterå *et al.*, 1998), there is growing concern and evidence that retaining a tag or recovery from tagging procedures can bias the physiology and behaviour of a fish (Bégout *et al.*, 2012). For example, external tags such as T-bar anchor tags are easy to attach and to distinguish, mostly inexpensive, but permanently penetrate and extrude from the skin, which may provide an access route for infection and over time, fouling of the tag may add weight and increased drag to the fish (Thorstad *et al.*, 2001). On the other hand, internal tags, such as passive integrated transponder (PIT) tags and data storage tags (DSTs), require an

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invasive, sub-dermal procedure to implant the tag (with particularly negative effects if the tag-to-body mass ratio is too high), yet small internal tags have been shown to stay in fish for years without any detectable effects (Jepsen *et al*., 2002; Drenner *et al*., 2012). Internal tags are not commonly visible externally, whereby detection and identification of tags require either specialized equipment or surgical removal of the tag to retrieve its data. Both external and internal tags may be lost during experiments; external tags may become loose and be lost at any time, while internal tags have the highest risk for expulsion shortly after tagging (Jepsen *et al*., 2002).

An alternative method of individual identification is to keep track of natural marks of individuals, such as fingerprints in humans, markings around the dorsal fins of killer whales *Orca orca via* photo-identification (Würsig & Jefferson, 1990), spot patterns in whale sharks *Rhincodon typus* Smith 1828 (Arzoumanian *et al*., 2005) and manta rays *Manta* spp. (Town *et al*., 2013) and scale patterns in common carp *Cyprinus carpio* L. 1758 (Huntingford *et al.,* 2013). In Atlantic salmon *Salmo salar* L. 1758, Garcia de Leaniz *et al*. (1994) found that small individual parr (44–66 mm) could be successfully identified from images taken 8 weeks before. Identification of these fish was based on patterns of very small melanophores (melanin-filled pigment spots) in the region of the eye and the jaw. In a follow-up study, Donaghy *et al*. (2005) proved that *S. salar* fry could be identified from these patterns at least 16 months later, when the fish were still relatively small (<110 mm). These patterns are absent in larger *S. salar* and therefore Merz *et al*. (2012) opted for the relatively large melanophore spot patterns on the dorsal head region to identify individual Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792). At the beginning of the study when the fish were on average 70 mm long ($n = 295$), there were no apparent spot patterns on their heads, but when the fish had grown to about 122 mm, the authors found clear spot patterns in *c*. 40% of individuals and could use these patterns to correctly identify fish 2 months later when they had grown to an average length of 139 mm.

*Salmo salar* is one of the most studied fish species due to its importance in aquaculture, commercial fisheries and recreational fishing. These studies encompass a range of fields spanning biology, physiology, genetics and ethology and often require a form of identification at the individual level. The aim of the current study was to document the development of melanophore spots in *S. salar* to determine if visual recognition of spot pattern could be used to identify fish after substantial growth; in this case, identifying large (*c*. 3 kg) *S. salar* individually in sea cages, based on images taken 10 months before.

## MATERIAL AND METHODS

### EXPERIMENTAL ENVIRONMENT AND ANIMALS

The experiment was conducted at the Matre Research Station of the Institute of Marine Research, western Norway. *Salmo salar* (AquaGen strain, $n = 1200$) were reared in six seawater tanks (3 m diameter, 6 $m^3$). When the fish were 12 months old (26 January 2011; hereafter the time point labelled as 12 months), a subsample ($n = 246$) were randomly netted out across the six tanks and anaesthetised using MS-222 (0·1 g $l^{-1}$). These fish were weighed (mean ± S.D. 98·5 ± 20·0 g) and measured for fork length ($L_F$, 209 ± 12 mm), then externally tagged adjacent to their dorsal

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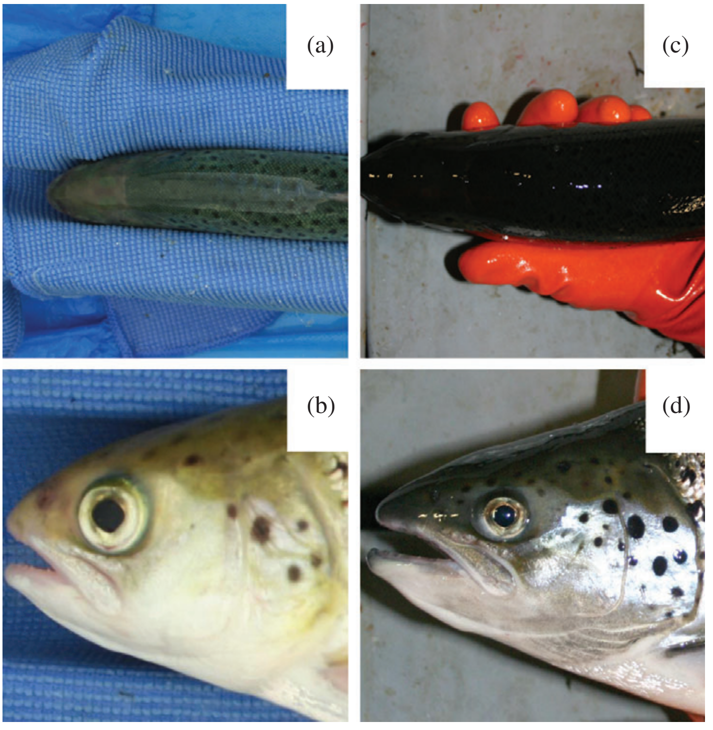


FIG. 1. Example images of the head of the same *Salmo salar* at (a), (b) age12 months and (c), (d) 22 months old viewed from (a), (c) above and (b), (d) the left side.

fin with 20 mm T-bar anchor tags that were uniquely coded with a four-digit number. Fish were then photographed (using a standard single-lens reflex camera mounted to a frame; Canon EOS 550D; www.canon.com) from a dorsal and left-lateral angle [Fig. 1(a), (b)] before being transferred back into the tank. This subsample formed the experimental group.

Two days later, the fish from all six tanks were transferred to nearby sea-cage facilities and reared in a single sea cage (125 $m^3$). At a second time point when the fish were 22 months old (29 November 2011), all fish were retrieved from the sea cage and euthanized with a lethal dose of anaesthetic. Fish with T-bar anchor tags were photographed in the same manner as at 12 months, again from the dorsal and left-lateral angle. At 22 months, mean (± S.D.) $L_F$ was 579 ± 92 mm and live mass ($M$) was 3088 ± 829·5 g. At 22 months, the dorsal skin colour of the large fish was too dark to be able to visually distinguish any spots [Fig. 1(c)]. It was established, therefore, that identifications of individuals in this study would instead be based on the overt spots on the operculum [Fig. 1(b), (d)]. Fish with no spots on the left operculum at 12 months (21 of 246 fish) were not used in the following recognition tests.

## EVALUATION OF SPOT DEVELOPMENT

As an initial proof of concept and in order to formulate a hypothesis on how to use the spot patterns for identification, a subset of pictures ($n = 6$) was evaluated to determine


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if opercular spots present at 12 months were still present at 22 months, if the spots had changed appearance (in terms of size and position) and if more spots had developed at 22 months. It was evident that spots present at 12 months did not disappear, but rather became larger and appeared as the most prominent at 22 months (Fig. 2, Supporting information). It was also evident that the total number of spots was considerably higher at 22 months, but as the spots that were present at 12 months looked to be the largest spots at 22 months, it was hypothesized that spot patterns could be used for long-term identification of *S. salar* (Fig. 2). This was then systematically tested through visual recognition of fish by participants provided with a single 12 month image of an individual and identifying that individual from a range of photos from 22 months; participants matching multiple individuals from multiple images at both 12 months and 22 months; automatic point-pattern matching of opercular spots, whereby an automatic script compared an individual's spot patterns at 12 months with spot patterns from multiple individuals at 22 months. In the first two tasks, there were three participants in the panel who were all fish biologists, but unfamiliar with opercular spot pattern identification. The relationship between fish size and the number of spots was also investigated.

*Visual recognition of one individual from multiple options*

The aim of this task was to determine whether a fish could be recognized at 22 months from multiple images, using a single photo taken at 12 months. Images from 12 and 22 months were standardized to the same size ($250 \times 250$ pixels) and to show only the head [Fig. 1(b), (d)]. Working independently, each participant was provided the same image of a fish at 12 months (randomly selected by the automated script; function random in Matlab; MathWorks Inc.; www.mathworks.com). The participants were then shown images of multiple fish at 22 months (also randomly selected by an automated script, but of which one had to be the 22 months image of the individual in the 12 months image) and given the task of choosing the image that matched their target individual. This task was repeated three times per participant with new 12 and 22 months images each time and with an increasing number of images to choose from: the number of 22 months photos provided were $n = 5$, 7, 9, 11, 13, 15, 20, 25 and 30.

*Visual pairing of individuals*

This task tested the ability to recognize individuals from a different approach. Participants were provided with a number of photos of fish at 12 months (randomly selected by the automated script) and their corresponding photos at 22 months, shuffled. The task was to take individuals at 12 months and pair the image with their respective 22 month image. Here, participants had the advantage of being able to first choose the simplest couplings. This setup, however, also meant that by default, one wrong pairing also resulted in another wrong identification. Again, the task was repeated with new groups of photos with an increasing number of photos provided: $n = 5$, 7, 9, 13, 15, 20, 25 and 30.

*Recognition by point pattern matching*

This test was conducted on the same images as the visual recognition of one individual from multiple options task, but as the point pattern method needs at least three points in order to compare angles between vectors, individuals with less than three


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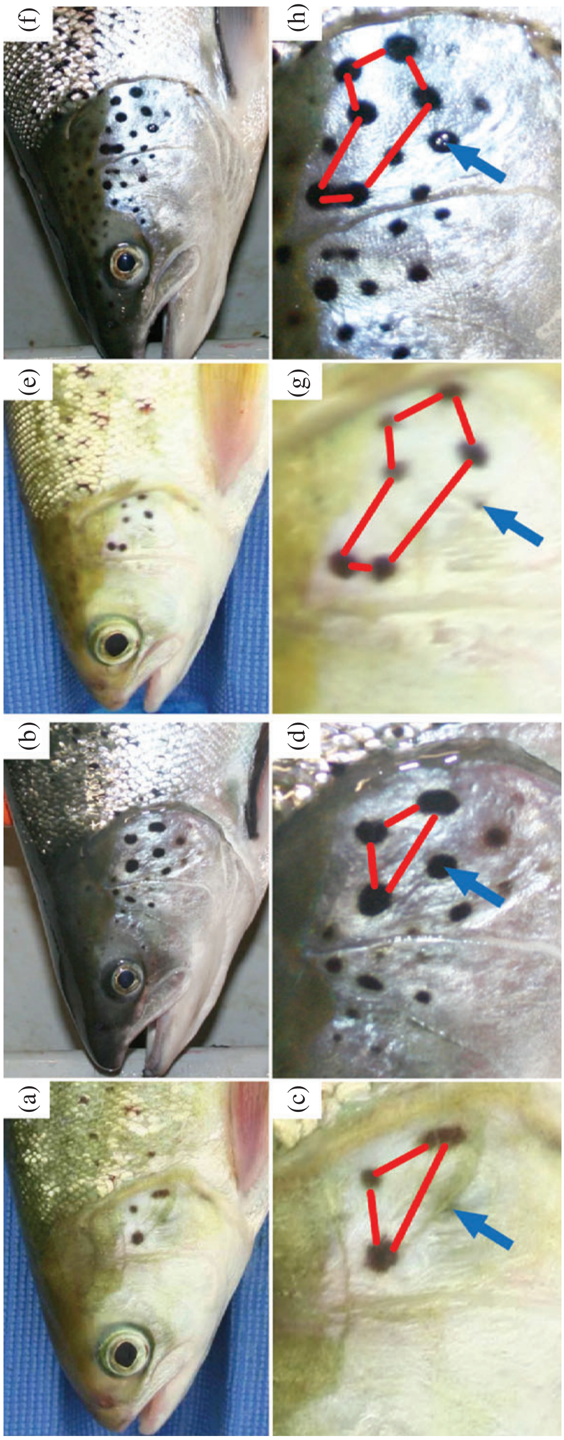


FIG. 2. Example of spot patterns on two individual (a), (b) and (e), (f) *Salmo salar* at (a), (e) 12 months and (b), (f) 22 months old. (c) and (d) show magnified view of operculum corresponding to (a) and (b); similarly, (g) and (h) show magnified view of operculum corresponding to (e) and (f). ——, Lines linking spot patterns developed at 12 months that are still present at 22 months, with these spots now being the most prominent, although more spots have arisen. ——▲, Spots that are barely visible on 12 month old fish, but appear as large spots at 22 months of age.

spots in the 12 month images were excluded. First, the coordinates of the central pixel of the spots in each of the 12 and 22 month images were manually identified, selecting the largest spot as the first for each 22 month image, under the assumption that this spot must also have been present at 12 months [Fig. 3(a), (b)]. Each of the coordinate sets from the images constituted a point pattern [Fig. 3(c), (d)] as input to an automatic script for matching star patterns (based on Murtagh, 1992; see following description). The script provided a score for each 12–22 month image combination and the scores were then ranked to determine which 22 month photo best matched the original fish at 12 months. As in the first task, the programme was provided an increasing number of 22 month images to choose from: $n = 5$, 7, 9, 11, 13, 15, 20, 25 and 30. Pseudocode of script programmed in Matlab: for each point *i* in the 12 month point pattern, set point *i* to be the field of view coordinate and create a vector $V_i$ containing the polar coordinates ($r$; $\theta$) as seen from point *i* and sorted by the amplitude of the angle $\theta$ [Fig. 3(e)]. Then for each 22 month point pattern, construct from the first point a vector $V_A$ of the polar coordinates of the other $m$-1 points of this point pattern represented by their polar coordinates [Fig. 3(f)]. $V_A$ is then downsized to $n - 1$ [Fig. 3(g)] by subsequently selecting the points nearest (Euclidian distance) the equivalent point in $V_i$ [Fig. 3(h)] and the difference between the $x$–$y$ coordinates of $V_i$ and $V_A$ calculated. To allow for a slight miss alignment of the patterns, the script performed this operation for angle translations of $V_A$ between $-5°$ and $+5°$, with an increment of 1°. After this first approximation of the correct sub-point pattern in the post-smolt point-pattern set and its corresponding $V_i$ vector, the process is rerun, but this time with the $r$-values calibrated so that the largest $r$ in each set is 1. Finally, the script returns the average Euclidean distance between the corresponding points in the calibrated 12 and 22 months coordinate sets. The assumption being that the distance between the 12 months coordinate set and the coordinate set of the same fish at 22 months would be smaller than the distances found between the 12 month coordinate set and the coordinate sets of the other 22 month fish in the sample.

### STATISTICS

Data analyses were performed using R software 2.9.0 (www.r-project.org). Number of spots at 12 and 22 months were compared by paired *t*-test (function t.test in R) and aggregated values as mean ± S.E. The relationships between fish size and the number of spots were analysed using generalized linear models (function glm in R) with quasi-Poisson distribution for count data with over-dispersion (Crawley, 2007). Consistency of variance and normality of the residuals were confirmed with model checking plots (function plot in R).

## RESULTS

### NUMBER OF SPOTS IN RELATION TO FISH SIZE

There was a clear increase in number of spots at 22 months compared with the number of spots at 12 months (*t*-test, 18·1 ± 0·5 *v*. 3·1 ± 0·2, $t_{245} = -35·1$, $P < 0·001$). The number of spots counted on the opercula at 12 months varied from 0 to 16 and from 2 to 37 at 22 months. There was no significant relationship between body mass and number of spots at either 12 months (GLM, $t_{244,245} = 1·8$, $P > 0·05$) or at 22 months (GLM,


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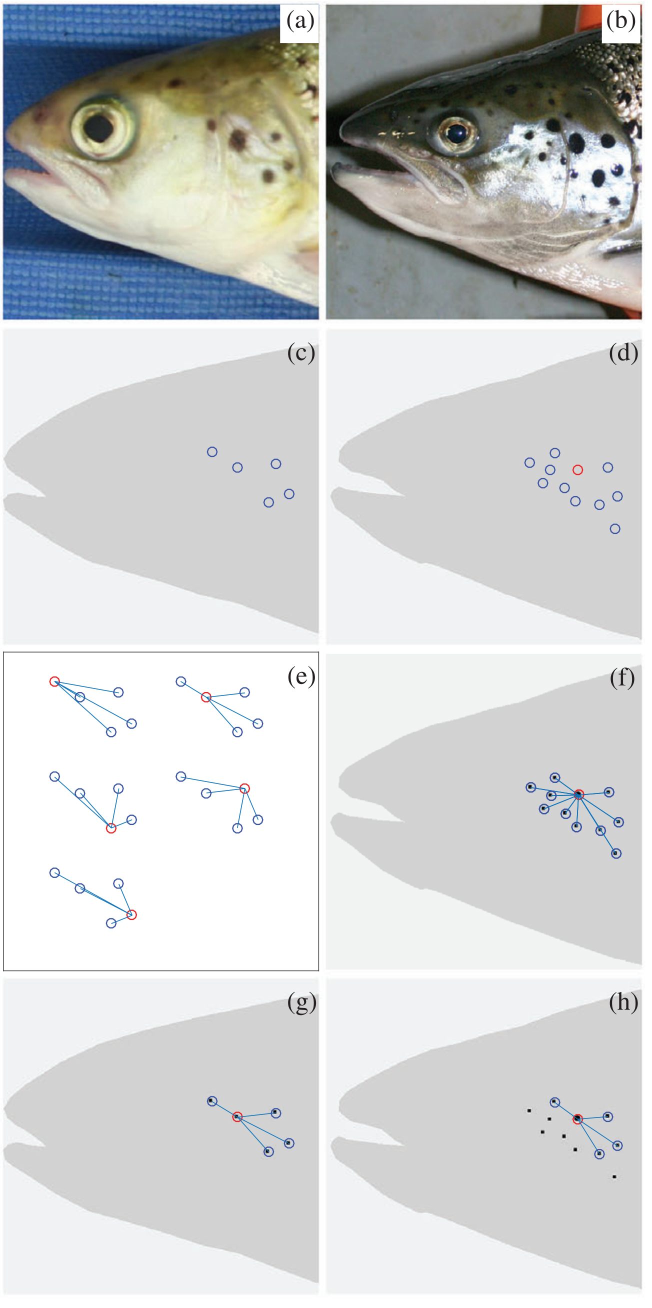


FIG. 3. Point pattern matching. (a) Original image of *Salmo salar* at 12 months old and (b) the same salmon at 22 months old. (c) The central pixel (○) marking the $x$–$y$ coordinate of each spot seen in (a) and similarly for (d) and (b), but where the dominant spot (○) is identified. (e) Possible ($r$,$\theta$) vector sets $V_i$ (/) based on the co-ordinates from (c): ○, the field of view coordinate. (f) Vector set $V_A$ (/) based on the coordinates from (d): ○, the field of view coordinate of the spot marked to be the most prominent spot in (d). (g) Vector set (/) with closest match to sub-vector set in (f). (h) The sub-vector set (/) in (f) with the closest match to the vector set in (g).

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TABLE I. Number of opercular spots in relation to *Salmo salar* body size at ages 12 and 22 months and adult sampling M22

| 12 Months | | | | | | 22 Months | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot class | Sample size $n$ | $L_F$ (cm) Mean | $L_F$ (cm) S.D. | $M$ (g) Mean | $M$ (g) S.D. | Spot Class | Sample size $n$ | $L_F$ (cm) Mean | $L_F$ (cm) S.D. | $M$ (g) Mean | $M$ (g) S.D. |
| 0 | 21 | 20·7 | 1·5 | 95 | 19 | 0–5 | 10 | 58·4 | 5·8 | 3135 | 956 |
| 1 | 45 | 20·6 | 0·9 | 93 | 13 | 6–10 | 27 | 59·3 | 3·6 | 3229 | 676 |
| 2 | 51 | 21·0 | 1·3 | 99 | 21 | 11–15 | 59 | 56·7 | 11·5 | 2963 | 918 |
| 3 | 49 | 20·7 | 1·3 | 97 | 21 | 16–20 | 56 | 56·9 | 8·7 | 3061 | 896 |
| 4 | 30 | 20·9 | 1·2 | 101 | 21 | 20–25 | 53 | 58·6 | 8·8 | 3105 | 745 |
| ≥ 5 | 50 | 21·3 | 1·2 | 104 | 22 | ≥ 26 | 41 | 59·3 | 10·1 | 3183 | 786 |

$L_F$, Fork length; $M$, live wet mass.

$t_{244,245} = 28·2$, $P > 0·05$) (Table I). There was, however, a trend for the mean number of spots to increase with $L_F$ at 12 months (GLM, $t_{244,245} = 1·8$, $P < 0·05$) and more so at 22 months (GLM, $t_{244,245} = 364·0$, $P < 0·01$).

## VISUAL RECOGNITION OF ONE INDIVIDUAL FROM MULTIPLE OPTIONS

When participants had to match one 12 month image with a 22 month image from multiple images of 22 month fish, 23 out of 27 fish (85%) were identified correctly by all three panel members (Table II), with two of the four remaining fish incorrectly identified by two or all panel members. In total, the correct 22 month fish was identified in 74 of 81 challenges (91%). All the wrongly identified fish had as few as three or less opercular spots in the 12 month images (Table II). Even so, two of the three participants managed to correctly identify both the two cases that had only one spot at 12 months. There was no obvious relationship between the number of 22 months image options ($n$) and correct identification.

TABLE II. Result for the three members of the panel (A, B and C) in identifying the correct image of a 22 month old *Salmo salar* in a set of $n$ 22 month images from an age 12 month image. The number of identification spots of the 12 month image is also given

| | Image sample size ($n$) of 22 month old fish | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | | | 7 | | | 9 | | | 11 | | | 13 | | | 15 | | | 20 | | | 25 | | | 30 | | |
| | Number of opercular identification spots per 12 month old fish | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Panel member | 5 | 6 | 2 | 4 | 4 | 5 | 5 | 1 | 7 | 3 | 2 | 3 | 4 | 1 | 6 | 8 | 2 | 7 | 2 | 2 | 3 | 3 | 3 | 7 | 4 | 4 | 5 |
| A | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| B | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| V | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |

■, Correct identification; □, wrong identification.

Table III. Result for the three panel members (A, B and C) in pairing the correct individual *Salmo salar* from $n = 5$, 7, 9, 11, 13, 15, 20, 25 and 30 images from 12 and 22 month old fish. The number of identification spots at 12 month is also given

| Image sample size (*n*) | | Correct identifications |
|---|---|---|
| 5 | Spots | 2 3 4 4 4 |
| | ABC | + + + + + |
| 7 | Spots | 2 3 3 4 5 6 8 |
| | ABC | + + + + + + + |
| 9 | Spots | 1 1 1 2 2 2 3 3 8 |
| | ABC | 1 1 + + + + + + + |
| 11 | Spots | 1 2 2 3 4 4 5 6 7 9 9 |
| | ABC | + + + + + + + + + + + |
| 13 | Spots | 1 1 1 2 2 3 3 3 4 4 4 5 6 |
| | ABC | − + 2 + 1 + 2 2 + + + + + |
| 15 | Spots | 1 1 1 2 2 2 2 3 3 4 4 4 4 5 8 |
| | ABC | 2 + 2 2 2 + 2 + + 2 + + + + + |
| 20 | Spots | 1 1 1 2 2 2 3 3 3 5 6 6 6 7 7 7 8 9 9 12 |
| | ABC | + + + + 2 + + + + + + + + + + 2 + + + 2 |
| 25 | Spots | 1 1 1 1 2 2 2 2 2 3 3 3 3 3 3 3 3 4 4 4 4 4 5 7 16 |
| | ABC | + + + 2 + + + + + 2 + 2 + + + + + + + + + + + + 2 |
| 30 | Spots | 1 1 1 2 2 2 2 2 2 2 3 3 3 3 3 4 4 4 4 4 4 4 4 5 6 7 8 9 10 12 |
| | ABC | + + + + 2 2 + + + + + 2 2 + + 2 + + + + + + + + + + + + + + |

−, None of the panel paired this fish correctly; 1, one of the panel paired this fish correctly; 2, two of the panel paired this fish correctly; +, all three panel members paired this fish correctly.

## VISUAL PAIRING OF INDIVIDUALS

When the task was to pair 12 and 22 month photos for numerous individuals, the participants were generally successful at correctly identifying the same fish at the second time point (93% correct pairings; Table III). When calculating the identification ratio according to number of spots at 12 months, there was a clear increase in identification success from few to many spots: 82, 90, 93, 97, 100, 100, 94, 100 and 100% from one to nine spots, respectively. In this task, there were very few individuals with more than nine spots (Table III). When the number of fish in the group was more than $n = 9$, there was a rise in the proportion of fish incorrectly matched, but there was only one case in 135 where all three participants failed to correctly pair the photos and one other case where only one participant correctly paired the photos (Table III).

## RECOGNITION BY POINT PATTERN MATCHING

When scoring all 22 month images for their Euclidian difference value, 17 out of 20 cases (across all *n* groups) were correctly the closest match to the original 12 month fish (Table IV). Of the three remaining cases, two ranked the correct image as the second-best match and the last case reported the correct fish as the third-best match (Table IV).


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TABLE IV. The sorted scores from the point matching algorithm comparing one image of a 12 month old *Salmo salar* with *n* images from 22 month old fish; the lower the score, the better the match. Bold indicates correct match values.

| Image sample size (*n*) of 22 month old fish | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | | 7 | | | 9 | | 11 | | 13 | | 15 | | 20 | 25 | | | 30 | | |
| Number of opercular identification spots per 12 month old fish | | | | | | | | | | | | | | | | | | | |
| 5 | 6 | 4 | 4 | 5 | 5 | 7 | 3 | 3 | 4 | 6 | 8 | 7 | 3 | 3 | 3 | 7 | 4 | 4 | 5 |
| **0·61** | **0·28** | **0·32** | 0·79 | **0·56** | **0·61** | **0·70** | 0·00 | **0·60** | **0·32** | **0·64** | **0·40** | **0·35** | 0·40 | **0·06** | **0·13** | **0·50** | **0·33** | **0·47** | **0·53** |
| 0·63 | 1·32 | 0·77 | **0·93** | 1·11 | 0·83 | 1·30 | **0·00** | 1·45 | 0·85 | 0·76 | 0·80 | 0·72 | 0·79 | 0·18 | 0·18 | 1·05 | 0·39 | 0·51 | 0·68 |
| 1·18 | 1·33 | 1·26 | 1·01 | 1·30 | 0·90 | 1·32 | 0·01 | 1·73 | 0·89 | 0·79 | 1·01 | 0·81 | **0·84** | 0·21 | 0·24 | 1·06 | 0·49 | 0·55 | 0·80 |
| 1·85 | 1·68 | 1·40 | 1·55 | 1·53 | 0·96 | 1·74 | 0·04 | 2·28 | 0·94 | 0·88 | 1·15 | 0·95 | 0·87 | 0·35 | 0·25 | 1·07 | 0·53 | 0·70 | 0·84 |
| 3·01 | 2·25 | 1·47 | 1·70 | 1·69 | 1·41 | 1·88 | 0·04 | 2·56 | 0·95 | 0·91 | 1·26 | 1·11 | 0·87 | 0·47 | 0·45 | 1·10 | 0·53 | 0·84 | 0·85 |
| | | 1·55 | 2·02 | 2·84 | 1·46 | 1·96 | 0·05 | 2·58 | 1·01 | 1·51 | 1·52 | 1·17 | 0·91 | 0·72 | 0·54 | 1·24 | 0·55 | 0·94 | 0·90 |
| | | 3·56 | 4·19 | 2·87 | 1·68 | 2·05 | 0·05 | 2·96 | 1·15 | 1·60 | 1·60 | 1·24 | 0·95 | 0·75 | 0·66 | 1·30 | 0·56 | 1·03 | 0·95 |
| | | | | | 1·78 | 2·20 | 0·07 | 3·08 | 1·20 | 1·70 | 1·74 | 1·35 | 1·01 | 0·86 | 0·82 | 1·34 | 0·56 | 1·14 | 0·96 |
| | | | | | 2·03 | 2·52 | 0·68 | 3·52 | 1·45 | 1·84 | 1·84 | 1·45 | 1·07 | 0·87 | 0·84 | 1·35 | 0·61 | 1·14 | 0·97 |
| | | | | | | | 0·88 | 4·89 | 1·64 | 2·35 | 1·91 | 1·48 | 1·20 | 0·90 | 0·99 | 1·38 | 0·90 | 1·15 | 0·97 |
| | | | | | | | 0·96 | 4·97 | 1·72 | 2·95 | 1·93 | 1·73 | 1·22 | 1·04 | 1·01 | 1·41 | 0·96 | 1·15 | 0·98 |
| | | | | | | | | | 1·86 | 3·14 | 2·00 | 1·76 | 1·33 | 1·19 | 1·03 | 1·42 | 0·96 | 1·15 | 1·04 |
| | | | | | | | | | 3·08 | 5·45 | 2·04 | 1·77 | 1·33 | 1·21 | 1·05 | 1·59 | 1·01 | 1·15 | 1·04 |
| | | | | | | | | | | | 2·14 | 1·77 | 1·41 | 1·44 | 1·07 | 1·62 | 1·05 | 1·37 | 1·17 |
| | | | | | | | | | | | 2·57 | 2·23 | 1·58 | 1·45 | 1·16 | 1·62 | 1·21 | 1·39 | 1·22 |
| | | | | | | | | | | | | | 2·04 | 1·51 | 1·17 | 1·66 | 1·22 | 1·41 | 1·30 |
| | | | | | | | | | | | | | 2·11 | 1·52 | 1·21 | 1·69 | 1·50 | 1·46 | 1·33 |
| | | | | | | | | | | | | | 2·13 | 1·53 | 1·28 | 1·72 | 1·51 | 1·47 | 1·50 |
| | | | | | | | | | | | | | 2·77 | 1·69 | 1·48 | 1·72 | 1·62 | 1·48 | 1·50 |
| | | | | | | | | | | | | | 2·81 | 1·75 | 1·73 | 1·76 | 1·72 | 1·50 | 1·53 |
| | | | | | | | | | | | | | | 2·06 | 1·77 | 1·79 | 1·77 | 1·61 | 1·55 |
| | | | | | | | | | | | | | | 2·28 | 2·48 | 1·99 | 1·85 | 1·76 | 1·69 |
| | | | | | | | | | | | | | | 2·30 | 3·28 | 2·33 | 2·17 | 1·90 | 1·74 |
| | | | | | | | | | | | | | | 2·69 | 3·70 | 2·69 | 2·29 | 2·03 | 1·80 |
| | | | | | | | | | | | | | | 2·91 | 7·39 | 3·23 | 2·30 | 2·11 | 1·83 |
| | | | | | | | | | | | | | | | | | 2·32 | 2·18 | 2·00 |
| | | | | | | | | | | | | | | | | | 2·34 | 2·41 | 2·21 |
| | | | | | | | | | | | | | | | | | 2·40 | 2·52 | 2·31 |
| | | | | | | | | | | | | | | | | | 2·72 | 2·98 | 2·34 |
| | | | | | | | | | | | | | | | | | 5·36 | 3·27 | 3·15 |

The image was the same one used in the trial in Table II, but excluding 12 month images with less than three spots.

## DISCUSSION

With the experiments and group of fish tested in this study, it was possible to recognize *S. salar* based on opercular spot patterns, even after a 30 fold increase in mass. As few as two spots and even only one spot, at 12 months was in many cases adequate for the majority of the assessor participants to pair the 12 month image to the correct 22 month image (Table II). Two spots are too few to be a pattern, indicating that the participants also used other clues such as position of the spots on the operculum, spot size and spot shape. This is in line with Huntingford *et al*. (2013), who found that volunteers could visually identify before and after images of *C. carpio* taken 3 months apart, without having any defined method for the identification.

When following a subgroup of individuals in a larger population under experimental conditions, researchers are likely to seek to identify one individual within a group. The results from the identifying one individual from multiple options task suggest that having four or more spots on the operculum at 12 months was enough for certain identification at 22 months (Table II). In the current case, that meant 32% of the fish at 12 months had enough spots on their left operculum for certain identification. Number of spots increased over time; at the 22 month sampling 98% (244 of 246 fish) had four spots or more. Therefore, a higher percentage of the fish would have had a sufficient number of spots for certain identification if the first photos had been taken later when the fish were larger. It is also likely that the certainty of correct identification would have increased if photographs of the right operculum at 12 and 22 months also had been available. The original intent of this study, however, was to use images of the dorsal head region for pattern recognition, as in Merz *et al.* (2012); consequently pictures were not taken of both opercula during sampling.

In an experimental setting, an alternative common practice would be the visual identification of all individuals in a group; for instance, those belonging to a treatment group. This would be similar to the visual pairing of individuals task in this study. Here, the instances when incorrect pairings were made by the participants were when they had fewer than three opercular spots on which to base their identification (Table III). This increases the number of individuals at 12 months with enough spots to 52%. Having the advantage of being able to first match the simplest couplings is therefore a clear advantage compared with having to identify one from multiple options. All participants incorrectly identified an individual only once, where the fish had only one spot at 12 months (Table III). Thus, more than one assessor decreases the risk of wrong identifications, as individuals where participants disagree on the identification should be disregarded from the trial. With only one assessor, however, wrong identifications could be minimized if individuals with uncertain identifications were classified as unidentified and disregarded from the trial. Even though participants performed well also when there were many images to compare, the risk of wrong identifications and the time spent processing images will inherently increase with number of individuals. Based on the current tests, therefore, it is recommended to use only visual recognition of individuals in experiments where the number of individuals is manageable, where the fish have more than three clearly distinguishable spots on at least one of their opercula and that images should be taken of both left and right opercula.

In *O. tshawytscha*, Merz *et al.* (2012) opted for melanophore spot patterns on the dorsal head region to identify individuals. The authors found 42% of the 254 fish in the trial formed identifiable patterns, 40% developed spots but patterns were insufficient for identification and 18% exhibited complete lack of spots. In their case, all fish with identifiable patterns were correctly recognized at four subsequent samplings (up to 140 mm fish length) using manual visual recognition of spot patterns. Dorsal spot patterns were therefore the initial choice of the present study, but since this region was very dark on the large (22 month) fish it was impossible to use this trait to pair the small (12 month) with the large (22 month) fish. Alternatively, the operculum had a silvery background colour, which provided contrast to the dark pigmentation spots. As with the top of the head, the operculum also has the advantage that it is a well-defined area that does not have scales that could affect the shape of the spots or that could be lost and leave darker areas that can be confused with spots. *Salmo salar* can occasionally develop shortened opercula over time due to genetic disposition or negative welfare (Pettersen *et al.,* 2014), which would render identification with opercular spots impractical. In these rare cases, the fish should be withdrawn from the subsample, just as an individual that has lost its external tag would be.

The tested point-pattern-matching script only uses the position of the spots in relation to each other in order find how close the spot patterns match. The results were seldom a perfect match (score = 0). This is probably due to the change in head shape as the fish grew [*i.e.* became more elongated; Fig. 3(a), (b)], which changes the vectors of the spot coordinates. The script could be improved by also utilizing information about spot size and position on the operculum as part of the algorithm. Ideally, the algorithm should also account for changes in the shape of the head during significant growth between identifications; further understanding of how head shape changes with fish size is required to facilitate the improvement of automatic point pattern matching identification. Nevertheless, this study used the largest spot on the operculum as a definite view point of the algorithm, which was sufficient to identify the correct individual in 17 of 20 tests (Table IV). The use of programme-driven processing of images is less demanding of time and labour than manual identification. In the future, it is hoped to improve the presented script and test alternative algorithms and available software solutions, *e.g.* the Sloop software (Duyck *et al.*, 2015) general pattern retrieval engine for individual animal identification.

Recognizing individual animals has been the cornerstone of husbandry in agriculture: identifying sick individuals or those that behave abnormally facilitates their treatment or removal. This is a difficult task in fish production, as farmers observe their animals using cameras and cannot easily move between or follow individual fish in a school. There are systems in development for visual recognition of individual cattle (Kumar *et al.*, 2016) and sheep (Yang *et al.*, 2016). If developed specifically for salmonid aquaculture, automated recognition systems could lead the industry towards a stage that is similar to that towards which agriculture is moving, *i.e.* precision livestock farming (Banhazi *et al.*, 2012). Production methods are enhanced by the ability to identify animals at the individual level, rather than perceiving and treating the farmed group as a single entity (Berckmans, 2014). Automatic identification of individuals would be a major advancement in aquaculture, where information on health, welfare, growth and social interactions could be assessed at a finer scale. For this to become reality does however require considerable improvements in automated recognition techniques of *S. salar* individuals. As of now individual recognition of *S. salar* will have its best use in controlled trials with few individuals, where it may have an important role, providing individual growth and behaviour data without risking unwanted effects from tagging.

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## Supporting Information

Supporting Information may be found in the online version of this paper:
**Video S1.** Opercula spots at 12 months vs at 22 months.

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